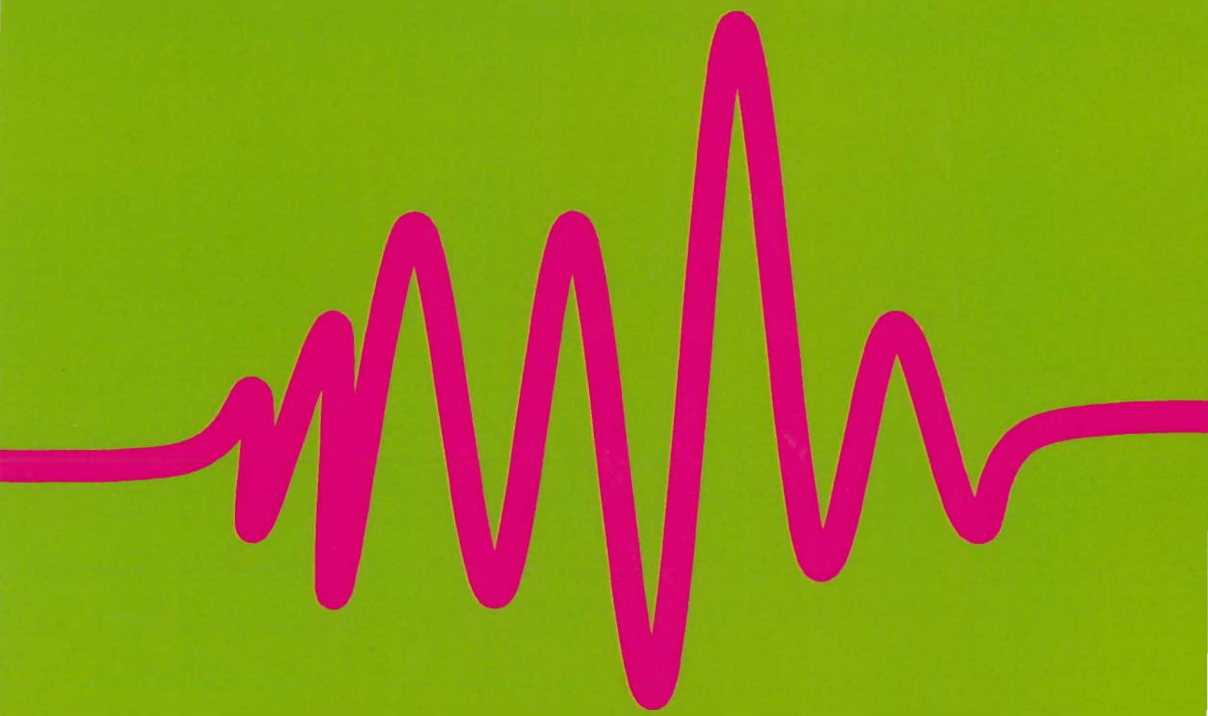
AMPLITUDE INTEGRATED EEG

Longitudinal recordings in critically ill newborns



Henk ter Horst

Amplitude integrated EEG

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Stellingen
behorend bij het proefschrift

amplitude integrated EEG

longitudinal recordings in critically ill newborns

Henk ter Horst
25 mei 2011

Het registreren van het aEEG is niet alleen nuttig bij pasgeborenen die een perinatale asfyxie hebben doorgemaakt. *Dit proefschrift.*

Bij kinderen met een congenitale hartafwijking wordt met behulp van het aEEG een indruk verkregen van de mogelijke hersenschade opgelopen in de preoperatieve fase. *Dit proefschrift.*

Het niet onder controle krijgen van epileptische aanvallen bij pasgeborenen met een ernstige perinatale infectie is een prognostisch ongunstig teken. *Dit proefschrift.*

Indien het aEEG te kort geregistreerd wordt, gaat belangrijke informatie verloren. *Dit proefschrift.*

Het is van essentieel belang te weten welke factoren het aEEG, hoe en wanneer, beïnvloeden. *Dit proefschrift.*

In de neonatologie is niets doen vaak moeilijker dan iets doen.

Give a man a fish, and he'll eat for a day, teach him how to fish and he'll eat forever.

Emancipate yourselves from mental slavery, none but ourselves can free our minds. *Redemption song, Bob Marley*

Commercialisering van het hoger onderwijs vormt een gevaar voor het vrije denken.

Een eerlijke positie op de wereldmarkt zal de derde wereldlanden meer gezondheidswinst brengen dan het sturen van dokters.

Onze afhankelijkheid van fossiele brandstoffen is nadelig voor de vrijheid van veel mensen.

De grens van behandeling van premature pasgeborenen moet niet gebaseerd zijn op de technische mogelijkheden.

Het is maar een proefschrift.

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Amplitude integrated EEG

Longitudinal recordings in critically ill newborns

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
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op gezag van de
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CHAPTER 1

Introduction

Introduction

Neonatal intensive care has improved enormously in recent years. Treatment strategies and equipment have become available for the most vulnerable infants. Survival has improved, even for the most premature infants. But short term and long term morbidity is still high (1). Efforts are made to improve survival as well as long term outcome.

Along with improvement of care for newborn infants, continuous monitoring of vital signs, such as blood pressure, and arterial oxygen saturation, has become part of routine neonatal care. Over the past decades attention has been drawn to the need for monitoring of brain function (2,3). Bedside methods are now available to continuously monitor brain function. Electro cortical activity can be recorded through a cerebral function monitor (CFM), which displays the so-called amplitude integrated electroencephalogram (aEEG) (4,5). A method to measure cerebral oxygenation is near infrared spectroscopy (NIRS).

Amplitude integrated EEG

aEEG is recorded through 2 biparietal electrodes. The positions used are P3 and P4 according to the international 10-20 system. This one channel EEG is converted into the aEEG by filtering, smoothing and rectifying. The signal is then compressed in time (6 cm/h) and displayed in a semi-logarithmic scale. The filter that is used attenuates frequencies below 2 Hz and above 15 Hz, and therefore minimizes artifacts. Because of the semi-logarithmic scale, brain activity of low amplitude is enhanced. Several features of the aEEG can be observed, these include the background pattern, sleep wake cycling and electrographic seizure activity. Analysis of the background pattern is done by pattern recognition. It represents the overall electro-cortical activity, and several patterns can be distinguished. These patterns are shown in Figure 1. Background pattern is highly dependent on gestational age, being more discontinuous at lower gestational ages. The second feature that can be observed is sleep-wake cycling (SWC). In term infants, SWC normally develops within the first day after birth. Finally the aEEG can be used to monitor electrographic seizure activity.

CFM was first introduced to monitor brain activity in adults. It was initially introduced into neonatal intensive care in Lund, Sweden. In the nineties it was introduced in the Netherlands, at first in Utrecht. In Groningen the first newborns were monitored using CFM in 1998. Nowadays all neonatal intensive care units in the Netherlands use CFM to monitor brain activity.

At first CFM was predominantly used to monitor brain activity in term infants that had had perinatal asphyxia. An impaired perfusion or oxygenation of the brain can lead to depression of brain activity. It is possible to predict neurological outcome on the basis of the background pattern (6-8). In case of low voltage background patterns (FT, CLV or BS) following perinatal asphyxia, outcome is poor.

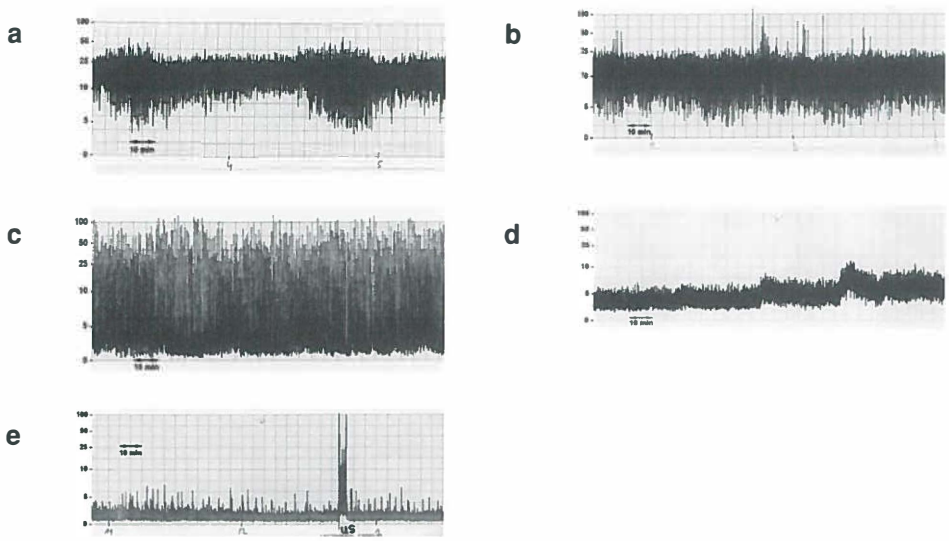


Figure 1: Examples of normal and abnormal aEEG patterns. The scale on the Y-axis is semi-logarithmical, i.e. linear from 0-10 μV, and logarithmical from 10 to 100 μV. The X-axis represents time, a 10 min period is indicated by the horizontal arrows. 1a. Continuous normal voltage with identifiable sleep stages (CNV-S). Continuous cortical activity with voltage 10-25 (50) μV; sleep cycling is signified by the band of aEEG activity altering in width. 1b. Discontinuous normal voltage (DNV). Discontinuous trace, where cortical activity is predominantly above 5 μV. 1c. Burst suppression (BS). Discontinuous background pattern, with periods of very low activity (<5 μV) intermixed with bursts of higher amplitude. 1d. Continuous low voltage (CLV). Continuous background pattern of very low voltage (around or below 5 μV). 1e. Flat tracing (FT). Mainly inactive (isoelectric) tracing of extremely low voltage, below 5 μV. The sudden increase of cortical activity is an artifact caused by performing a cranial ultrasound, as indicated by the marking US, administered by the nursing staff.

Following perinatal asphyxia seizures may also evolve, and these seizures are often subclinical. Electrographic seizure activity can be monitored with the aEEG, apart from background activity. It is also possible to evaluate the efficacy of treatment with anti-epileptic drugs. It is believed that ongoing seizure activity is damaging to the brain, especially to the immature brain (9,10). Whether subclinical seizures should be treated is still under debate. A recent study showed that longer duration of electrographic seizure activity leads to more severely MRI abnormalities, suggesting that prompt treatment of seizures, both clinical and subclinical, may prevent brain injury (11). Whether the treatment of subclinical seizures eventually also leads to less severe impairment, remains to be answered.

With respect to monitoring of seizures the technique has some limitations. Non experienced users may miss a substantial amount of electrographic seizure activity (12). Short (less than 30 seconds) and focal seizures are missed using CFM (13). On the other hand, clinical judgment is also fairly unreliable (14). A large amount of electro-

graphic seizure activity is subclinical, and a large amount of what we, clinicians, believe to be clinical seizures are not accompanied by electrographic seizure activity (15). Round the clock continuous video-EEG monitoring is not available. Therefore CFM, to date, is the most optimal monitor for neonatal seizure detection. With the introduction of digital CFMs it has become possible to analyse the single channel EEG, on which the aEEG is based. This may add to the proper interpretation of the aEEG.

Near Infrared Spectroscopy

NIRS is a non-invasive method that measures regional cerebral oxygen saturation (r_cSO_2). R_cSO_2 reflects the oxygen saturation in a mixed vascular bed dominated by venules. This technology is based on the fact that biological tissue is relatively transparent to near-infrared light (600 to 900 nm). The optical sensor measures the quantity of reflected light photons as a function of two wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue (16,17). NIRS differentiates oxygenated hemoglobin from deoxygenated hemoglobin that has distinct absorption spectra. The ratio of oxygenated hemoglobin to total hemoglobin reflects the regional oxygen saturation of tissue. By using two detectors at a distance of 3 and 4 cm from the near-infrared optode, it is possible to differentiate between more superficial tissues and cerebral tissue. The detector placed at 3 cm from the optode receives light scattered predominantly from scalp and skull. The detector placed at 4 cm receives light scattered from scalp, skull, and cerebral tissue. Thus, by subtraction, the two detectors measure the oxygen saturation in the underlying cerebral tissue.

The fractional tissue oxygen extraction (FTOE) can be calculated from r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$) (18). It reflects the balance between oxygen delivery and oxygen consumption and may thus indicate cerebral hypoxemia or ischemia. NIRS has only been introduced in neonatal care recently. There are many conditions that may be accompanied by impaired cerebral perfusion or oxygenation. Examples are: hypotension; a persistent open ductus arteriosus with a large left to right shunt; hypocarbia; etc. Since NIRS reflects the balance between oxygen delivery and consumption it may be a promising technique to identify infants at risk of developing brain injury

Depression of electro-cortical activity may be preceded by diminished cerebral perfusion (19,20). An increase of the FTOE may indicate a diminished cerebral perfusion. On the other hand, if the FTOE is within high or normal ranges and is accompanied by low electro-erebral activity, this may indicate low cerebral metabolism because of neuronal death (21). The combination of both techniques may enable us to gain more insight in the evolution and timing of brain injury and may help to guide the treatment of infants that are at risk of brain injury.

Objective

The objective of this thesis was to investigate the longitudinal course of aEEG patterns in critically ill newborns, both term and preterm, and whether this course was related to clinical circumstances.

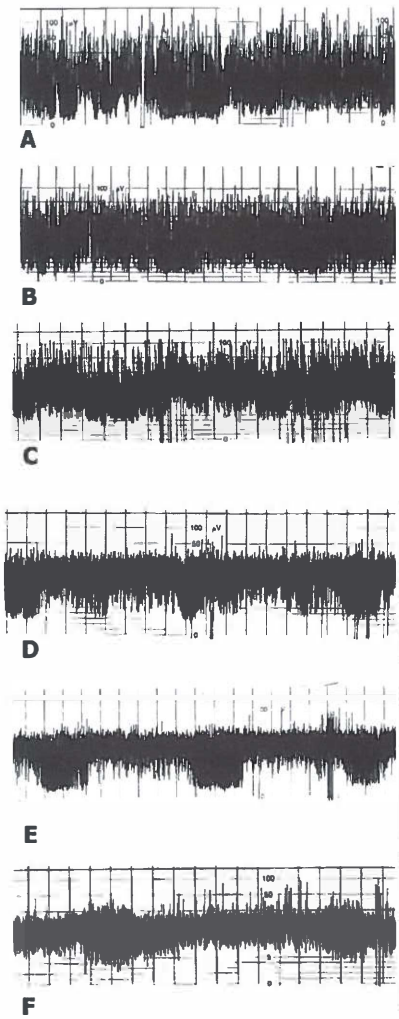
Outline of the thesis
This thesis consists of three parts.

Part 1. Amplitude integrated EEG recordings in critically ill term newborns
C*hapter 2* is about the evolution of aEEG following severe perinatal asphyxia. The course of the aEEG was analysed for the first 72 hours after birth. We investigated whether normalisation of aEEG was related to good outcome. Another group of infants that is at risk for developing brain injury are infants with a neonatal sepsis or meningitis. In *chapter 3* the predictive value of the aEEG in this group of infants was studied. Another group of infants at risk for neurodevelopmental delay, is the group of infants that undergo surgery because of a congenital heart disease (22). The timing of brain injury is many fold (23). Injury may originate prior to surgery, e.g. at the time when following closure of the ductus arteriosus oxygen delivery to the brain is impaired. It may even develop *in utero*. We studied aEEG in term infants with congenital heart disease prior to surgery in c*hapter 4*.

Part 2. Amplitude integrated EEG recordings in preterm newborns
Electro cerebral activity in preterm infants is predominantly discontinuous, and is influenced by gestational and postnatal age (24,25). Based on features of the aEEG of preterm infants, Burdjalov designed a new scoring system (Table 1 en Figure 2).

Table 1: CFM Score according to Burdjalov (24)

Score	Continuity	Cycling	Amplitude of Lower Border	Bandwidth Span and Amplitude of Lower Border
0	Discontinuous	None	Severely depressed (<3 μV)	Very depressed: low span (15 μV) and low voltage (5 μV)
1	Somewhat continuous	Waves first appear	Somewhat depressed (3–5 μV)	Very immature: high span (>20 μV) or moderate span (15–20 μV) and low voltage (5 μV)
2	Continuous	Not definite, somewhat cycling	Elevated (>5 μV)	Immature: high span (>20 μV) and high voltage (>5 μV)
3		Definite cycling, but interrupted		Maturing: moderate span (15–20 μV) and high voltage (>5 μV)
4		Definite cycling, noninterrupted		Mature: low span (<15 μV) and high voltage (>5 μV)
5		Regular and mature cycling		



Co	Cy	LB	B	T
0	0	1	1	2 24-25 weeks

Co	Cy	LB	B	T
1	1	2	2	6 27-28 weeks

Co	Cy	LB	B	T
2	2	2	2	8 29-30 weeks

Co	Cy	LB	B	T
2	3	2	3	10 31-32 weeks

Co	Cy	LB	B	T
2	4	2	3	11 34 weeks

Co	Cy	LB	B	T
2	5	2	4	13 36-37 weeks

Figure 2: On the left side aEEGs with progression of postconceptional age are shown; on the right the scoring system according to Burdjalov (24).

Therefore the aEEG recordings in preterm infants have limited predictive value for the long term outcome. An exception may be infants with large intraventricular hemorrhages (26). Other factors may influence aEEG and should be accounted for when using aEEG in preterm infants. We introduce a new method of analysing the aEEG in *chapter 5*. This method enables us to analyse the aEEG in more detail. We also investigated the influence of illness severity and low blood pressure, besides effects of gestational and postnatal age. In *chapter* 6 we studied the relationship between electro cerebral activity and regional cerebral oxygen saturation in preterm infants.

Part 3. Pitfalls in amplitude integrated EEG recording

aEEG is routinely used as a bedside monitor in high risk newborns in most Dutch neonatal intensive care units. The main objectives are to monitor overall electro-cerebral activity and to detect electrograhic seizure activity. Based on the background pattern, together with neuro-imaging, and physical examination, conclusions on long term prognosis are often drawn. Therefore it is very important to know the limitations of the technique. Factors influencing the aEEG may alter the predictive value of aEEG. One of the factors that should be accounted for is medication. We discussed the effect of midazolam in *chapter 7*. With the introduction of the digital CFM's it has become possible to analyse the single channel EEG which forms the basis of the aEEG. We report on the value of this simultaneous recording in *chapter 8*.

We conclude this thesis with a general discussion and future perspectives.

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PART 1

Amplitude integrated EEG recordings in critically ill term newborns

CHAPTER 2

Prognostic significance of amplitude-integrated EEG during the first 72 hours after birth in severely asphyxiated neonates

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Pediatric Research 2004, 55:1026-1033

Abstract

Amplitude-integrated EEG (aEEG) is used to select patients for neuroprotective therapy following perinatal asphyxia, because of its prognostic accuracy within several hours after birth. We aimed to determine the natural course of aEEG patterns during the first 72 h of life, in relation to neurological outcome, in a group of severely asphyxiated term infants. Thirty infants, admitted to our neonatal intensive care unit from October 1998 until February 2001, were studied retrospectively. The aEEG traces obtained during the first 72 h after birth were assessed by pattern recognition: continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage (CLV) and flat trace (FT). Epileptic activity was also determined. The course of aEEG patterns was examined in relation to neurological findings at 24 mo. Initially, 17 of 30 infants had severely abnormal aEEG patterns (BS or worse), which changed spontaneously to normal voltage patterns (CNV, DNV) in 7 within 48 h. The sooner the abnormalities on aEEG disappeared, the better the prognosis. The likelihood ratio of BS or worse for adverse outcome was 2.7 (95% CI 1.4-5.0) between 0 and 6 h and increased to a highest value of 19 (95% CI 2.8–128) between 24 and 36 h; after 48h it was not significant. Normal voltage patterns (CNV and DNV) up to 48h of life were predictive for normal neurological outcomes (negative likelihood ratios <0.3). Our findings indicate that the course of aEEG patterns adds to the prognostic value of aEEG monitoring in asphyxiated infants. Spontaneous recovery of severely abnormal aEEG patterns is not uncommon.

Introduction

Birth asphyxia is a major cause of perinatally acquired brain injury in full-term infants, accounting for approximately 20-30% of all cases of cerebral palsy (1, 2). Several methods are used to assess the extent of neuronal damage during the first days and weeks of life. These include clinical signs (3), neuro-imaging (4, 5) and neuro physiological techniques (6-9). During the first few hours after birth, continuous amplitude-integrated EEG (aEEG) recorded with a cerebral function monitor is one of the most accurate bedside methods to establish neurological prognosis in term asphyxiated infants (10, 11). The aEEG records a single channel EEG from biparietal electrodes; frequencies < 2Hz and >15Hz are filtered selectively, and the amplitude of the signal is integrated. The processed signal is recorded semilogarithmically onto a printer with a low paper speed (12). Previous studies have shown that aEEG correlates well with conventional EEG (13, 14).

Because of its prognostic accuracy very soon following perinatal asphyxia (80-85% within 6h after birth), aEEG is the most suitable method to identify groups eligible for potentially neuroprotective treatments (15), e.g. brain cooling (16, 17) and oxygen radical scavenging (18). There are, however, only a few studies that investigated the longitudinal course of aEEG tracings during the first few days following severe perinatal asphyxia in the absence of such interventions (7, 12). It is therefore necessary to obtain data on the 'natural' course, i.e. without any neuroprotective intervention, of aEEG tracings in severely asphyxiated infants.

The aim of our study was to examine the prognostic accuracy of the course of aEEG tracings during the first 72 h after birth in a group of full-term infants who presented with severe perinatal asphyxia. Specific questions we addressed were as follows: (1) Which longitudinal courses of aEEG recordings can be identified during the first 72h in term infants following severe perinatal asphyxia? (2) What is the relationship of these courses with neurological outcome at 2 y of age? (3) What is the role of conventional EEG, in relation to aEEG recordings and neurological outcome?

Methods

Patients

We performed a retrospective cohort study. From our medical records we identified all term infants (gestational age 37-42 weeks), born between October 1998 and February 2001, admitted on the first day of life to the neonatal intensive care unit (NICU) of the University Hospital Groningen, the Netherlands, and treated for severe perinatal asphyxia. Severe asphyxia was defined if at least 2 of the following conditions were present:

(1) Signs of fetal distress (late decelerations on fetal heart rate monitoring or meconium staining of the amniotic fluid)
(2) Umbilical pH or first capillary pH (within 30 min after birth) < 7.00
(3) Delayed spontaneous respiration, necessitating artificial ventilation at 5 min.
(4) Signs of multi-organ-failure.

Neonates with major congenital malformations and chromosomal abnormalities were excluded. The study was approved by the Institutional Review Board, and informed consent was obtained from the parents.

The cohort consisted of 39 infants. In four infants aEEG recordings were not done, and in another four infants the aEEG recordings lasted < 12 h. One infant died at 19 h of age because of severe cardiac failure, and he was also excluded from further analysis. As a result, the study population consisted of 30 infants for whom reliable aEEG recordings had been performed continuing after 24h of age.

Clinical data of the study population are shown in Table 1. Most of the infants were outborn. Birth weights ranged from 2200 to 4445 g (median 3485 g), Apgar scores at 1 min from 0 to 6 (median 1) and at 5 min from 0 to 8 (median 4). Two infants had birth weights below the fifth centile. Initial pH values ranged from 6.65 to 7.22 (median 6.86) with base excess from –32 to –11.6 (median –22.1). From the medical records we obtained additional data on the obstetric history, stage of encephalopathy according to Sarnat and Sarnat (3) at 24h of age, presence of seizures and antiepileptic treatment, and neuro-imaging data. Although not an entry criterion for the study, all infants had at least mild encephalopathy, stage I. In three infants, the stage of encephalopathy could not be assessed because of the use of paralysing agents (Table 1). Clinical seizures occurred in 23 infants, in 17 infants already before the aEEG recording had been started. All infants with clinical seizures were treated with anti-epileptic drugs (phenobarbital as drug of first choice). During aEEG recording, clinical seizures occurred in 11 infants, 6 of whom had not yet received anti-epileptic drugs.

Eight infants died in the neonatal period. Follow-up of the other infants was regularly performed at the outpatient clinic and consisted of a paediatric and a neurological examination, based on Touwen (19). The paediatricians involved in the follow-up of our NICU infants were not aware of the aEEG findings. Follow-up data are available on all infants at the age of 24 mo. The neurological findings were classified as normal, severely abnormal (severe mental and motor delay, infantile spasms or cerebral palsy according to the criteria of Hagberg et al (20)), and mildly abnormal (neurological abnormalities present, other than cerebral palsy or infantile spasms, e.g. gross motor abnormalities, co-ordination problems, epilepsy or hearing deficits).

Assessment of aEEG recordings

Recording of the aEEG (Cerebral Function Monitor 4640, Lectromed, Ltd., Letchworth, UK) was started immediately after admission, at a median age of 4h 35 min after birth (range 60 min - 22h). The median duration of aEEG recordings during the first 72h after birth was 44h 33 min (12h 30 min - 68h). The aEEG was recorded from biparietal needle electrodes, and displayed on the integral printer at 6 cm/h. The impedance between electrodes was also recorded, and was always below 5 kΩ. Handling of the infant, observed clinical seizures and administration of anticonvulsant or sedative drugs were recorded by the nursing staff.

Table 1: Clinical data of the study group

No	GA (wk)	Birth Weight (g)	In/ Outborn	Obsteric complications	Signs of fetal distress	AS 1-5 min	Initial pH	Initial BE	Sarnat @ 24 h	Sz	AED	CUS	Outcome @ 24 mo
1	40,3	3410	Outborn	Prolonged labour	FHR	2 - 4	6,69	-22	II	Multiple	Phen, Fenyt, Clon	Slitlike ventricles, PVE (bright brain)	Died
2	41,3	3595	Outborn	Fetal-maternal transfusion	FHR	1 - 3	6,78	-24	II	Multiple	Phen, Fenyt, Clon	Slitlike ventricles, PVE	Mildly abnormal
3	37,4	2845	Outborn	Abruptio placentae	FHR	1 - 3	6,79	-29	II	Single	Phen	Normal	Normal
4	41,4	3560	Outborn	Prolonged labour	Mec	1 - 5	7,07	-15,6	II	Single	Phen	Normal	Normal
5	40,6	3470	Outborn	Perinatal infection (GBS)	None	5 - 5	6,88	-32	II	Multiple	Phen, Fenyt, Clon, Loraz	Slitlike ventricles, PVE (bright brain)	Died
6	41,9	3865	Outborn	Prolonged labour	FHR, Mec	2 - 6	6,93	-18	II	Multiple	Phen, Clon, Mdz, Lido,	Slitlike ventricles, PVE (bright brain)	Died
7	37,1	3250	Outborn	Unknown	Mec	0 - 5	6,73	-20,7	II	None	None	PVE	Mildly abnormal
8	41,4	4100	Outborn	Prolonged labour, shoulder dystocia	Mec	2 - 3	6,66	-25	Sed	None	Phen	Slitlike ventricles	Normal
9	39,7	3320	Outborn	Perinatal infection	FHR, Mec	3 - 5	6,77	-28	II	Multiple	Phen, Clon, Lido	Slitlike ventricles, PVE+THAL (bright brain)	Abnormal
10	41	3730	Outborn	Prolonged labour	FHR	1 - 6	6,77	-25	II	Single	Phen	Slitlike ventricles	Mildly abnormal
11	42	3540	Outborn	Umbilical cord intertwined	FHR, Mec	1 - 3	7,22	-15	II	Multiple	Phen, Clon	Slitlike ventricles, PVE+THAL (bright brain)	Abnormal
12	41,6	4250	Outborn	Prolonged labour	FHR, Mec	1 - 1	6,81	-26,4	III	Multiple	Phen, Clon, Mdz	Slitlike ventricles, PVE+THAL (bright brain)	Died
13	40	3420	Outborn	Prolonged labour	Mec	2 - 6	6,88	-24,6	II	Multiple	Phen, Fenyt	Slitlike ventricles	Mildly abnormal
14	38	3500	Inborn	Maternal shock	FHR	1 - 3	6,91	-17,5	II	Multiple	Phen	Slitlike ventricles	Normal
15	40,3	3260	Outborn	Perinatal infection (GBS)	None	1 - 4	6,73	-30	III	Multiple	Phen, Clon, Lido	Slitlike ventricles, PVE+THAL (bright brain)	Died
16	38	2400	Outborn	TTTS	None	3 - 4	6,84	-24	I	None	None	Slitlike ventricles	Normal
17	40	3255	Outborn	Umbilical cord intertwined	FHR	1 - 3	6,97	-23	II	Single	Phen	Slitlike ventricles	Normal
18	41,9	3965	Outborn	Prolonged labour, shoulder dystocia	Mec	2 - 3	7, 14	-11,6	II	Multiple	Phen, Clon	Slitlike ventricles, PVE	Normal
19	42	3580	Outborn	Prolonged labour	FHR, Mec	2 - 4	6,74	-24,3	I	Multiple	Phen, Clon	Slitlike ventricles	Mildly abnormal
20	38,7	2200	Outborn	Abruptio placentae	FHR, Mec	6 - 7	6,99	-14	Sed	None	None	Slitlike ventricles, PVE	Normal
21	41,4	3950	Inborn	Perinatal infection (GBS)	FHR, Mec	4 - 4	6,75	-24	Sed	Single	Phen	PVE	Normal
22	40	3180	Outborn	Prolonged labour	FHR, Mec	3 - 5	6,92	-19	I	None	None	Slitlike ventricles, PVE	Normal
23	38	2835	Outborn	Prolonged labour	FHR	1 - 3	6,90	-15,6	II	Multiple	Phen, Clon	Normal	Normal
24	38	2900	Outborn	Abruptio placentae	FHR	0 - 0	6,67	-22,1	III	Single	Phen, Clon	Slitlike ventricles, PVE+THAL (bright brain)	Died
25	40	4445	Outborn	Unknown	Mec	2 - 4	7,10	-11,6	II	Single	Phen	PVE	Normal
26	37,9	3200	Outborn	Abruptio placentae	FHR	0 - 4	7,04	-17,1	II	Multiple	Phen, Lido	Slitlike ventricles, PVE+THAL (bright brain)	Abnormal
27	42	4075	Inborn	Uterine rupture	FHR	0 - 5	6,65	-31	III	Multiple	Phen, Lido, Clon	Slitlike ventricles, PVE (bright brain)	Died
28	41,1	3780	Inborn	Prolonged labour	FHR	5 - 5	6,99	-14	I	None	None	Normal	Normal
29	39,1	3500	Outborn	Unknown	Mec	1 - 1	6,72	-20,2	III	None	None	Intraparenchymal Hemorrhage	Died
30	41,7	2770	Outborn	Unknown	FHR, Mec	0 - 4	6,92	-19	II	Multiple	Phen	Normal	Mildly abnormal

GA, gestational age; FHR, fetal heart rate; Mec, meconium; Sed, Sedated; Phen, phenobarbital; Fenyt, Fenytoin; Clon, clonazepam; Lido, lidocain; Mdz, midazolam; Loraz, lorazepam; PVE, periventricular echodensities; THAL, thalamic echodensities; GBS, Group B Streptococcus; BE, base excess; Sz, sezures; AED, Anti-epileptic drugs; CUS, cranial ultasound

We analysed the aEEG using pattern recognition according to the definitions given by Toet *et al* (11):

Continuous Normal Voltage with cycling of sleep stages (CNV-S): Continuous background activity with voltage 10-25 (-50) μV, with the band of aEEG activity altering in width, indicating cycling of sleep stages (21).
Continuous Normal Voltage (CNV): Continuous background activity with voltage 10-25 (-50) μV, but without sleep stages.
Discontinuous Normal Voltage (DNV): Discontinuous trace, with voltage predominantly above 5 μV.
Burst Suppression (BS): Discontinuous trace with periods of very low cortical activity (< 5 μV), intermixed with bursts of higher amplitude.
Continuous Low Voltage (CLV): Continuous background pattern of very low voltage (around or below 5 μV).
Flat Tracing (FT): Mainly inactive (iso-electric tracing) of extremely low voltage (< 5 μV).

Epileptic activity was also identified:
Epileptic Activity (EA): Single or repetitive events (at a frequency of less than once per hour), with sudden sustained cortical activity.
Status Epilepticus (SE): Repeated epileptic activity, resulting in a regular pattern of increased cortical activity (sawtooth pattern).
A CNV pattern was considered as normal, DNV as mildly abnormal, all others as severely abnormal. Examples of the various aEEG patterns are shown in Figure 1.

All traces were analysed off line by 3 investigators (HtH, CS, AFB), blinded to neurological outcome. It included an assessment of the background pattern, including epileptic status, and, if present, transitions from one pattern to another. A transition was regarded as such if the new pattern had lasted at least 30 min. The presence of isolated events of epileptic activity was also noted. Artefacts were excluded, unless they were very short-lasting and the aEEG pattern before the artefact was similar to the pattern afterwards. Agreement about the specific type of aEEG pattern was reached in 88% of cases (*kappa* = 0,85).

The sequence of patterns of each individual infant was graphically displayed on the time axis for the first 72 h of life, with the time of birth as t = 0. In this way, individual longitudinal sequences of aEEG patterns were obtained from each infant. Next, the longitudinal sequences of aEEG patterns were grouped according to neurological outcome.

In 24 cases a conventional EEG (20 electrodes, duration 1h) was obtained between 12 and 48h of life, simultaneously with recording of the aEEG. One clinical neurophysiologist (TvW), unaware of the aEEG findings and experienced in interpreting neonatal EEG, assessed all EEGs. The background pattern of the EEG was classified as normal, discontinuous, suppression-burst, low voltage, and isoelectric (22). In

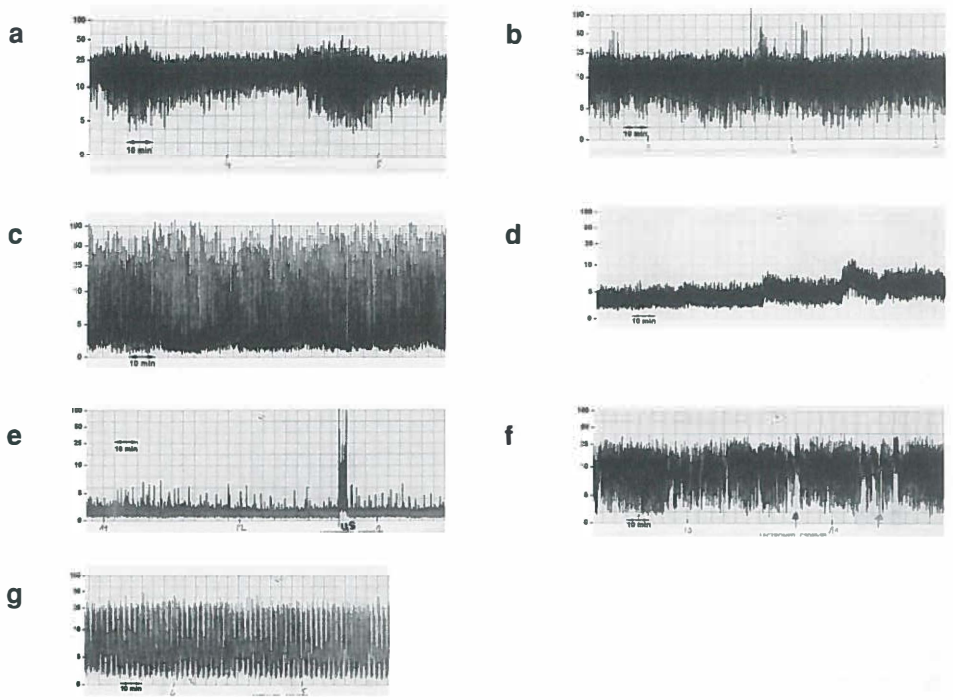

Figure 1: Examples of normal and abnormal aEEG patterns. The scale on the Y-axis is semi-logarithmical, i.e. linear from 0-10 µV, and logarithmical from 10 to 100 µV. The X-axis represents time, a 10 min period is indicated by the horizontal arrows. The numbers in some figures on the X-axis are markings from the medical and nursing staff, indicating hours on the first or second day after birth. 1a. Continuous normal voltage with identifiable sleep stages (CNV-S); 1b. Discontinuous normal voltage (DNV); 1c. Burst suppression (BS); 1d. Continuous low voltage (CLV). 1e. Flat tracing (FT); 1f. Continuous or discontinuous normal voltage with (suspected) epileptic activity (CNV / DNV +EA); 1g. Status epilepticus (SE).

addition, presence and location of epileptic discharges were noted. Spike-waves were classified as epileptic discharges. Spikes and sharp waves were considered to be epileptic if there was a repetitive character or if they showed a persistent focality. Sharp transients were not noted as an epileptic phenomenon. The findings on conventional EEG were compared with the findings on the aEEG trace, with respect to background patterns and presence of epileptic phenomena.

Statistical analysis

For testing the correlation between the degree of abnormality of the aEEG patterns and neurological outcome the Spearman Rank Order Correlation coefficient was calculated and two-tailed tested. Positive (LR+) and negative likelihood ratios (LR) were calculated to assess the predictive value of abnormal and normal aEEG traces for neurological outcome. A p-value < 0.05 was considered statistically significant.

Results

Longitudinal course of aEEG patterns

Seventeen infants had severely abnormal aEEG patterns at admission. Fourteen were admitted before 12 h after birth. In 5 of them the aEEG improved to normal voltage patterns (CNV, DNV) within 12 h after birth, in two it improved between 24 and 48 h after birth. In 10 infants the aEEG patterns remained severely abnormal throughout the first 72h of life or until death, although the degree of abnormality changed in 3 of them from low voltage patterns to BS.

Thirteen infants had normal (CNV, n = 6) or mildly abnormal (DNV, n = 7) aEEG traces at admission. In these infants, all traces remained of normal voltage, except for two infants. In one infant a short period of BS occurred between 24 and 36 h after birth; in the other infant EA was obvious at ~ 72h after birth.

Epileptic activity on aEEG

In the total group, EA was recognised on 10 aEEG traces, either as isolated events or as epileptic status. In 2 of these infants, the electrographic EA was not clinically evident at all, and in 4, it continued or returned electrographically after apparently successful treatment of the clinical manifestations. Suspicious clinical signs suggestive of seizures that could not be recognised on the aEEG traces occurred in 4 infants. All infants with either clinical or silent seizures were treated with antiepileptic drugs. Treatment with antiepileptic drugs never changed a normal pattern into a severely abnormal one, although in some infants, the pattern became transiently more discontinuous than before, for ~ 30 to 60 min.

The course of aEEG patterns in relation to neurological findings at 2 y

Figure 2 displays the longitudinal course of aEEG patterns recorded during the first 72 h after birth of all infants, grouped according to neurological outcome.

Thirteen infants were normal at follow up. All except 4 showed aEEG traces with normal voltage patterns (DNV, CNV, CNV-S). Three infants had short periods of burst suppression (BS) and one infant even a short period of low voltage trace (FT and CLV) at ~ 6 h after birth. All improved spontaneously to a normal voltage pattern (DNV, CNV, CNV-S) within the first 12 h after birth.

Six infants had mild neurological deficits at follow up. Their aEEG traces showed predominantly normal voltage patterns (DNV, CNV, CNV-S) as well. Two infants had additionally short periods of BS, one of them during the first 12 h after birth and the other between 24 and 48 h after birth. Another infant had a low-voltage pattern (CLV) followed by a long period of BS up to 36 h after birth, intermixed with short periods of clinical seizures and EA on aEEG. Finally, one neonate had initially a DNV trace, but got clinical seizures and EA on the aEEG on the third day of life.

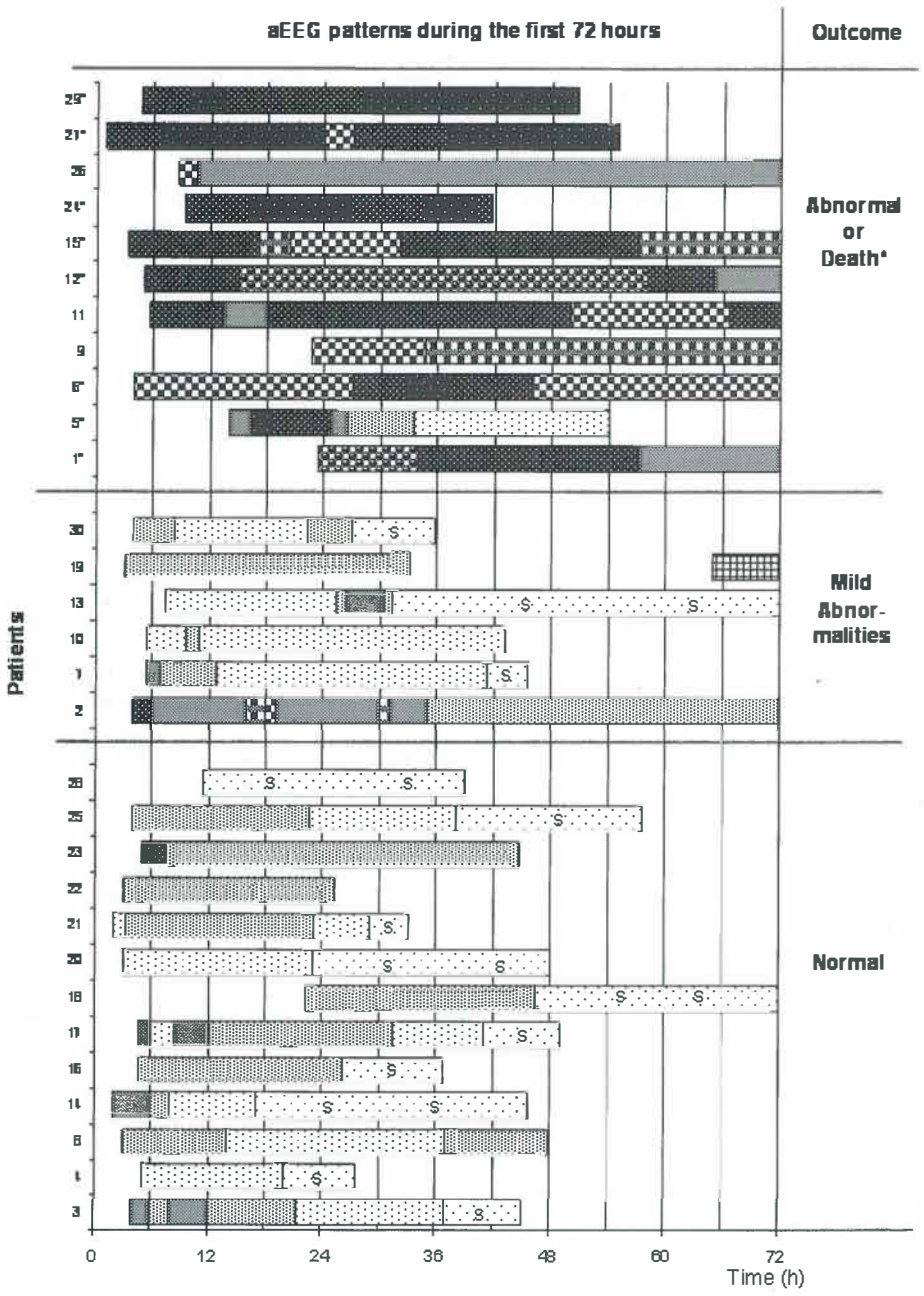

Figure 2: Longitudinal course of aEEG patterns in 30 term asphyxiated neonates, during the first 72 hours of life, grouped according to their neurological outcomes.

CNV–S: S; CNV: ; DNV: ; CNV / DNV + EA: ;BS: ;BS + EA: ;

SE: ;CLV: ;CLV + EA: ;FT: .

Three infants had severe neurological deficits at follow-up, and 8 infants died. All but one had severely abnormal aEEG patterns (BS, CLV, FT) throughout the first 72h after birth. Eight of them had in addition episodes of epileptic status (n = 6) or EA superimposed on CLV (n = 2).

Prognostic value of aEEG patterns for neurological outcome at 2 y

A strong correlation existed between the sequence of normal and severely abnormal aEEG patterns and neurological findings at follow-up (Table 2). The sooner the abnormal aEEG patterns normalised, the better the prognosis. Persistent abnormal aEEG patterns were predictive of severe deficits or death. The cut-off point was between 12 and 48 h after birth (Table 3). Patterns showing BS or worse had, if present before 6 h of age, a LR+ of 2.7 (95% confidence interval (CI) 1.4-5.0) for adverse outcome; it increased to a highest value of 19 (95%CI 2.8-128) between 24 and 36 h; after 48 h, it was not significant anymore. Normal voltage aEEG patterns (CNV, DNV) had LR- for severe neurological deficits at follow-up < 0.3, throughout the first 48 h of life.

Table 2. Correlation between the course of aEEG patterns and neurological outcome (only infants recorded within 12 hours were included)

	Neurological outcome at 24 months			
aEEG patterns	Normal	Mild deficits	Severe deficits or deceased	Total
Only DNV/CNV, including SWC	6	1		7
Only DNV/CNV, no SWC	2	1		3
DNV/CNV → BS/EA (→DNV/CNV)		2		2
FT/BS/EA → DNV/CNV before 12h of age	4	1		5
FT/BS/EA → DNV/CNV before 36h of age		1		1
Persistent FT/CLV/SE/BS			8	8
Total	12	6	8	26

Spearman correlation Coefficient: $r = 0.792$; $p < 0{,}001$ (two-tailed). The arrow (→) marks a transition from one pattern to another. **CNV**, continuous normal voltage; **DNV**, discontinuous normal voltage; **BS**, burst suppression; **CLV**, continuous low voltage; **FT**, flat trace; **SWC**, sleep wake cycling; **SE**, status epilepticus; **EA**, epileptic activity.

AEEG patterns did not differentiate between normal and mild deficits at follow-up. Out of this group of 19 infants, only 3 had exclusively CNV patterns; all developed normally. Sixteen infants had mildly abnormal aEEG patterns (DNV) or worse for at least some period of the study. Normalisation towards CNV and duration of these mildly

abnormal traces were not different between groups. Severely abnormal patterns (BS or worse) were observed in 7 infants, four of whom developed normally. Their aEEG patterns improved to CNV or DNV before 12 h of age. Improvement towards CNV or DNV occurred slightly later, between 24 and 36 h, in 2 of 3 infants with mild deficits. Cycling of sleep stages occurred in 10 of 13 later normal infants (median onset 28.5 h after birth), compared to 3 of 6 later mildly abnormal infants (median onset 32 h after birth). None of these differences did reach statistical significance. Absence of sleep cycling in case of normal voltage aEEG patterns was also not predictive for mild deficits (LR+: 2.2, 95%CI 0.6-7.8; LR-: 0.7, 95%CI 0.3-1.5).

Table 3. Positive (LR+) and negative (LR–) likelihood ratios (95% confidence intervals) for abnormal aEEG patterns (burst suppression or worse) from birth to 72h of age as predictive test for severe neurological abnormalities at 2 years of age.

	LR+ and LR– (95% confidence intervals)		
Postnatal age	LR+	LR–	N
0-6h	2.7 (1.4-5.0)	< 0.3	22
6-12h	3.6 (1.7-7.6)	< 0.2	26
12-24h	19.0 (2.8-128)	< 0.1	30
24-36h	9.5 (2.6-35)	< 0.1	30
36-48h	> 11	0.09 (0.01-0.59)	24
48-72h	3.6 (0.64-20)	0.15 (0.02-1.0)	13

Conventional EEG in relation to aEEG and neurological outcome

Conventional EEG was performed in 24 infants between 12 and 48 h of life. In the 6 infants in whom it was not performed, aEEG traces were exclusively CNV or DNV, and neurological outcome was normal. In the remaining infants, agreement of the type of background pattern between EEG and aEEG was 83% ($\kappa = 0.79$). Disagreement was diverse: One infant had CNV on aEEG, with a discontinuous background pattern on conventional EEG; one had DNV on aEEG with a normal background pattern on EEG. Two infants had CLV on aEEG, whereas conventional EEG was judged as suppression-burst in the one and isoelectric trace in the other.

Two infants had epileptic status/repetitive EA on aEEG that was confirmed by conventional EEG. In infants with normal voltage background activity, 2 of 12 had focal EA on conventional EEG, which was not identified on aEEG. At follow-up, one of those infants had mild neurological deficits and one was normal.

Discussion

The present study indicates that the natural course of aEEG patterns in term asphyxiated infants can be very diverse. Despite the profound severity of the asphyxia, 40-50% of infants had normal voltage aEEG patterns shortly after birth, even though it was discontinuous in the majority of infants. In those presenting with severely abnormal aEEG patterns, spontaneous recovery within 12 h toward normal voltage traces occurred in approximately one third of infants. Our data are in line with previous studies on conventional EEG, showing that normalisation of abnormal background patterns is not uncommon during the first day of life, and associated with normal neurological outcomes (22-24). Future studies investigating neuroprotective strategies should take this rate of spontaneous recovery into account.

Apart from asphyxia, antiepileptic drugs may also have contributed to the discontinuity of the EEG (8, 25). Previous studies have shown that this might occur in a small number of infants (7, 11). However, the suppressive effects of antiepileptic drugs on aEEG in most studies is reported as relatively short lasting, several hours at most (10), or absent (12). In the present study, none of the infants with normal voltage patterns developed severely abnormal patterns after antiepileptic treatment.

We found that aEEG at an early age differentiates very well between infants with later severe neurological deficits and infants with mild deficits or normal outcomes. When aEEG background patterns were of normal voltage (CNV, DNV) or normalised before the age of 24h, prognosis was fair, whereas severely abnormal aEEG patterns persisting beyond that age were related to adverse outcomes. Previous studies have shown that, already several hours after birth, aEEG recording is a reliable method for early prediction of neurological outcome in asphyxiated infants, with accuracy between 70 and 85% (8, 11, 12, 26). Our findings indicate that extension of the recording period up to 48h after birth adds to the prognostic value.

We were not able to detect a particular course of aEEG patterns that differentiated between normal infants and infants with later mild deficits. Severely abnormal aEEG patterns, if present at all, tended to improve slightly later in the group of infants with mild deficits, between 24 and 36 h instead of before 12h of age. However, the number of infants in this subgroup is too small to provide conclusive evidence in this respect. Normal (CNV) and mildly abnormal (DNV) aEEG patterns were predominant in both groups of infants, similar as has been reported before (11). Neither the presence of DNV patterns nor the age at which improvement of DNV into CNV occurred was different between groups. Presence or emergence of sleep stages did also not have any prognostic value. It must be noted that follow-up is relatively short, and it might be that neurological or developmental abnormalities appear later in life.

We included death and severe neurological abnormalities as a single outcome group. For determining the prognostic value of aEEG recordings, it is a practical classification, but it might be incorrect if clinical decisions to discontinue intensive care treat-

ment were made on the basis of aEEG recordings. Decisions that intensive care treatment is not appropriate should not be based on a single test but rather after thorough investigations, repeated clinical examinations and discussions between professionals have taken place, and with consent of adequately informed parents. The close relation between the aEEG recordings, the conventional EEG findings, the clinical and the neuroimaging data in both survivors and nonsurvivors of the poor outcome group indicate that discontinuing intensive care is unlikely to have biased our findings.

In this group of asphyxiated infants, 60% of the infants had suspicion of single or multiple clinical seizures and were treated with antiepileptic drugs. After treatment, aEEG patterns of several infants still revealed repeated EA, even epileptic status, which was clinically not obvious in several infants. It is widely known that electrographic discharges do not necessarily result in clinical seizures, before and after antiepileptic treatment (27). From previous studies it is estimated that > 50% of seizures identified on EEG or aEEG in newborn infants may be silent (28-30). There is considerable controversy over whether silent seizures in infants with hypoxic-ischemic encephalopathy lead to damage of the developing brain and should be treated (31). It might be argued that epileptic discharges in this setting are a consequence of damage that is already present, and therefore antiepileptic treatment will not influence later outcome. In addition, several anti-epileptic drugs administered in the perinatal period even induce neuronal apoptosis (32). On the other hand, animal and human data indicate that seizures in the developing brain may be harmful, at least at short term, considering disturbances in cerebral blood flow, energy metabolism and excitotoxic amino acids (33-36). Data on long term follow-up, however, are lacking. Further studies are required to elucidate this controversy and to optimise treatment in this respect.

There was a good agreement between conventional EEG and aEEG recordings, similar as in previous studies (8, 12, 14). Conventional EEG provided additional information in only 2 infants, showing focal EA that was not identified on aEEG. In general, seizure activity is well recognised on aEEG (26, 37). It is well known, however, that focal and short-lasting electrographic seizure activity (<30 s) is not detected by aEEG (14, 38).

The advantages of continuous aEEG recording in a NICU setting are many-fold. Interpretation is rather easy and can be learned by nurses, junior medical staff and neonatologists after a short period of training. It reveals background patterns and their changes, including epileptic status and (repetitive) electrographic seizures lasting > 30 s. It is useful as a monitoring and screening device for prolonged periods. We used needle electrodes, which were well tolerated without causing any harm. Similar to al Naqeeb *et al.* (26), we did not observe any complications related to its use. It is possible to perform a conventional EEG simultaneously. Thus, the aEEG may serve well as a screening test, and a conventional EEG can be performed on detection of abnormalities.

Conclusions

The present study confirms the prognostic accuracy of aEEG patterns shortly after birth in term asphyxiated infants. Our findings indicate that the course of aEEG patterns during the first 48 h after birth adds to the prognostic value of aEEG monitoring. Spontaneous recovery of severely abnormal aEEG patterns is not uncommon. The sooner the abnormalities on aEEG disappear, the better the prognosis.

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CHAPTER 3

The prognostic value of amplitude integrated EEG in neonatal sepsis and/or meningitis

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Abstract

Aim: To investigate the longitudinal course and prognostic value of amplitude integrated EEG (aEEG) in infants with neonatal sepsis or meningitis.
Methods: aEEG recordings of 22 infants with sepsis/meningitis were retrospectively evaluated. Mean gestational age was 38 weeks (range 34 - 42 weeks).Thirteen infants had meningitis. Survivors were seen for neurological follow-up. Four infants died, 2 were severely abnormal at 24 months. aEEG background pattern, sleep wake cycling (SWC) and electrographic seizure activity (EA) were appraised.
Results: All infants with continuous low voltage or flat trace on aEEG (n= 4) had an adverse outcome. Low voltage aEEGs (n= 9) had a positive LR (LR+) for an adverse outcome of 5.3 (95% CI 1.9-14.8) at 6 hours and 8.3 (95% CI 1.3-55) at 24 hours after admission. EA was more frequent in infants with adverse outcome ($p < 0.01$) and had a LR+ for adverse outcome of 10.6 (95% CI 1.5-76). SWC appeared more frequent in infants with good outcome ($p < 0.05$).
Conclusion: Low voltage background pattern, SWC and EA on aEEG are helpful to predict neurological outcome in infants with neonatal sepsis or meningitis.

Introduction

Neonatal sepsis has an incidence of 1-8 per 1000 live births. Neonatal meningitis appears less frequently with an incidence of 0.5-3.2 per 1000 live births (1). Group B streptococci (42%) and *Escherichia Coli* (16%) predominantly cause neonatal sepsis and meningitis. The mortality rate of neonatal meningitis is 10-15% (2). Survivors have neurological sequelae of varying degrees in 17-60 % of cases (1, 3).

The prognosis of neonatal sepsis and/or meningitis can be estimated by platelet and leucocyte count, endotoxin and interleukin 1 levels in the cerebrospinal fluid and the presence of coma (1). Mortality is associated with the presence of seizures, ventriculitis, hydrocephalus, coma, a gestational age below 33 weeks and a birth weight below 2000 g (2). EEG can be used to predict the neurological outcome in neonates and older children with a bacterial meningitis (4, 5).

In infants, following perinatal asphyxia, recording of the amplitude integrated EEG (aEEG) by a cerebral function monitor (CFM) is an easy and reliable method to predict the neurological outcome at a very early stage (6, 7). Furthermore it is a useful tool to monitor seizure activity and response to anti-epileptic drugs (AED). The aEEG is continuously recorded making use of biparietal electrodes. It reveals a background pattern of electro-cortical activity and shows seizure activity. Low voltage background patterns in asphyxiated infants are associated with a poor neurological prognosis. If the electro-cortical background of the aEEG is normal, there is a good chance that the infant will recover without sequelae. Previous studies have shown that the background pattern of the aEEG correlates well with conventional EEG (8, 9). As a monitoring tool for seizure activity there are limitations, short and focal seizures can be missed (9). When used by non-experts a substantial part of electrographic seizure may be missed (10). The proper interpretation of electrographic seizures can be enhanced by simultaneous use of single channel EEG recordings (11, 12). In future automatic seizure detection might improve the seizures yield (13).

The aim of our study was to investigate the course of the aEEG pattern in infants with neonatal sepsis and/or meningitis. A second aim was to determine the prognostic value of the aEEG in the same population. Our specific questions were 1) What is the relationship between the background pattern and the neurological outcome? 2) Is the presence of sleep wake cycling predictive for a normal neurological outcome? 3) Is the presence of epileptic activity related to neurological outcome?

Methods

We performed a retrospective study. We selected all infants who were admitted to the level III neonatal intensive care unit (NICU) of the University Medical Center Groningen, the Netherlands between January 2000 and January 2006, who were treated for neonatal sepsis and/or meningitis and had an aEEG recorded. In our NICU aEEG recordings were routinely performed in all critically ill infants. Recordings are continued for at least 12 hours following admission, but longer if abnormalities are present. aEEG recordings were interpreted by the attending neonatologist. Meningi-

tis was defined as a positive culture of cerebrospinal fluid (CSF) or an elevated cell count (> 20 x 10^6/l) in CSF with a negative culture. Sepsis was diagnosed by clear clinical deterioration, circulatory insufficiency, need for artificial ventilation and/or a positive blood culture.

Infants with a gestational age below 34 weeks were excluded. Twenty-four infants were identified. For one infant the recording lasted < 12 hours and for one infant aEEG recording was started more than 24 hours after admission. These two infants were excluded from the analysis. As a result the study population consisted of 22 infants of whom aEEG recordings were available. The Institutional Review Board approved the study.

aEEG recordings

Mean recording time was 45.3 hours (15-72). Biparietal needle electrodes (P3 and P4 position according to the international 10-20 system of electrode placement) were used for recordings; the impedance was less than 5 kΩ for the duration of each recording.

Assessment of aEEG recordings

aEEG was recorded by one of two cerebral function monitors, either the Lectromed® Multitrace 2 or the Olympic® CFM 6000. When the analog monitor (Lectromed® Multitrace 2) was used, it was calibrated every 24 hours. The nursing staff recorded all nursing and medical procedures, clinical seizures and the administration of all medications.

The aEEG recordings were assessed by pattern recognition. The background pattern, epileptic activity and presence of sleep wake cycling were appraised. When the aEEG was recorded on the digital monitor (Olympic® CFM 6000), epileptic activity was confirmed by analysis of the simultaneously recorded single-lead EEG. For classification of the different background patterns the criteria according to Toet et al. (6) were used:

Continuous normal voltage (CNV). Continuous background pattern with voltage 10-25 (50) μV.
Discontinuous normal voltage (DNV). Discontinuous background pattern with voltage predominantly >5 μV.
Burst suppression (BS). Discontinuous trace with periods of very low cortical activity (<5 μV), intermixed with bursts of higher amplitude.
Continuous low voltage (CLV). Continuous background pattern of very low voltage (around or below 5 μV).
Flat trace (FT). Extremely low voltage/isoelectric trace (<5 μV).

Epileptic activity was also appraised:
Electrographic seizure activity (EA). Single or repetitive events of sudden sustained high cortical activity.

Status epilepticus (SE). Repeated EA, resulting in a regular pattern of increased cortical activity (saw tooth pattern).

Sleep wake cycling (SWC). SWC was identified when there was an altering width of the amplitude of the tracing, indicating alternating different sleep stages.

CNV was considered a normal background pattern, DNV mildly abnormal. Low voltage traces (BS, CLV and FT) were regarded as severely abnormal. All traces were independently analysed off-line by 3 investigators (H.t.H., M.v.O., and H.R.). Investigators were blinded to the clinical course and the neurological outcome. Agreement regarding the specific type of background pattern was reached in 80% of cases ($\kappa = 0.7$). There was no disagreement regarding the presence of EA.

For each patient the longitudinal course of the aEEG pattern was displayed on a 72-hour time axis. The longitudinal courses were then grouped for outcome. (Fig. 1)

Statistical analysis

SPSS software for Windows, version 14.0 (SPSS Inc. Chicago, IL, USA) was used for all analyses. For testing the relation between aEEG background pattern and neurological outcome, the chi-square test for trend was used. Likelihood ratios were calculated to assess the predictive value of the background pattern and EA for the neurological outcome. Mean values were analysed by an independent-sample *t*-test in the case of a normal distribution or Mann-Whitney *U* in the case of a non-normal distribution. For testing the proportions of categorical data we used the Fisher's Exact test or the chi-squared test. A *p*-value < 0.05 was considered significant.

Results

Subjects

The clinical data of the study population are summarised in Table 1. The gestational age ranged from 34 to 42 weeks (mean 38 weeks) with birth weights from 1387 to 4405 (mean 3188 grams). The mean postnatal age at admission was 5.3 days (1-17). Fourteen infants had meningitis. The other eight infants had sepsis without meningitis. The infants with meningitis were significantly older than the infants without (2.7 versus 7.0 days; Student *t*-test, $p < 0.05$). Group B streptococcus was isolated in 14 cases (9 meningitis), *E.Coli* in 4 cases (3 meningitis). From the medical records we obtained additional information regarding presence of clinical seizures, neurological condition, antiepileptic therapy, the use of sedatives, the need for mechanical ventilation, the need for inotropes, laboratory results at admission, and neuro-imaging data. One infant was neurologically normal during aEEG recording, three infants received neuromuscular blocking agents, 11 infants were hypertonic and irritable, and seven infants were hypotonic and lethargic. Eighteen infants received morphine and/or AED. Twelve infants had clinical seizures, 19 infants were artificially ventilated and twelve infants were treated with inotropes. There were no differences in the 5 minute Apgar score, blood pH, blood lactate, blood pressure, platelet and leukocyte

Table 1: Clinical data of the study group

No	GA (wk)	Birth Weight (g)	AS 5	pH*	BE*	Lactate* (mmol/l)	CRP (g/l)	Platelet count (10^9/l)	Leucocyte count (10^9/l)	Men	Culture	Ino	MV	Sz	AED	CUS	Outcome
1	36.6	3100	7	7.23	-5	5	27	269	6	N	GBS	Y	Y	N	None	Normal	Normal
2	40	4405	9	7.23	-12	20.1	103	220	2.7	Y	GBS	N	Y	Y	Phen	Normal	Normal
3	40.4	4000	10	7.18	-7		296	99	3.1	Y	GBS	N	Y	Y	Phen,lido,mdz,clon	Increased PVE, vasculitis	SA
4	42	3495	6				78	133	16.6	N	Unkown	N	Y	Y	Phen	Normal	Normal
5	40.6	3360	5	6.88	-32		82	118	6.7	Y	GBS	Y	Y	Y	Phen,feny,clon	Oedema	Died
6	34.1	2600	9	7.37		12.8	297	5	1.8	Y	E. Coli	Y	Y	Y	Phen,lido,mdz, clon	PHI	Died
7	39.9	3860	9	7.34	0	2.5	5	386	13.6	Y	Viral	Y	Y	Y	Phen,clon	Normal	Normal
8	39	3190	9	7.17	-18		172	60	7.4	Y	GBS	Y	Y	Y	Phen,lido,mdz	Oedema	Died
9	37.3	2540	7	7.09	-16	12.4	156	39	30	Y	GBS	Y	Y	N	None	Normal	Normal
10	37.4	2450		7.29	-1		109	126	21.1	N	S.Aureus	N	Y	N	None	Normal	Normal
11	40	4045	10	6.92	-5	33.5	101	20	14.5	N	E. Coli	Y	Y	N	Phen,lido,mdz	Increased PVE	Died
12	34	1387	9	7.30	-6		79	17	11.9	Y	E. Coli	N	N	N	None	Normal	Normal
13	38.9	3300	10	6.65	-29	25	47	90	18.4	Y	GBS	Y	Y	Y	Phen,lido,mdz**, clon	Normal	Normal
14	40	3460		7.21	-6		234	28	14.1	Y	GBS	N	Y	Y	Phen,lido,clon	Increased PVE, ventriculomegaly	MA
15	34.4	2300	10	7.30	-2		120	217	2.1	Y	Str. Bovis	Y	Y	N	None	Normal	Normal
16	41	3850	7	7.22	-6	4.4	14	279	33.8	N	GBS	Y	Y	N	None	Normal	Normal
17	37.1	3590	9	7.47	-6	4.8	32	302	22.4	Y	GBS	N	Y	Y	Phen	Normal	Normal
18	39.3	3490		7.37	-3	4.1	164	263	13.7	Y	GBS	N	N	Y	Phen,mdz	Increased PVE	SA
19	38.6	3220	10	7.29	-7		90	50	24.5	Y	E. Coli	N	N	Y	Phen	Normal	MA
20	37.4	3640	10	7.25	-4		106	56	17.9	N	GBS	Y	Y	N	None	Normal	Normal
21	34.4	2235	10	7.38	-22		77	72	40.8	N	GBS	Y	Y	N	None	Normal	Normal
22	35.4	2640	8	7.19	-6		26	83	10.9	N	GBS	N	Y	N	Phen	Normal	normal

GA, gestational age; AS 5, Apgar Score at 5 minutes; BE, base excess; Me, meningitis; Ino, inotropes; MV, mechanical ventilation; Sz, seizures; AED, anti epileptic drugs; CUS, cranial ultrasound; Y, yes; N, no; GBS, group B streptococci; Phen, phenobarbital; Lido, lidocaïne; mdz, midazolam; feny, fenytoïn; clon, clonazepam; PVE, periventricular echodensities; SA, severely abnormal; MA, mildly abnormal. * measured at admission, ** midazolam treatment after aEEG recording

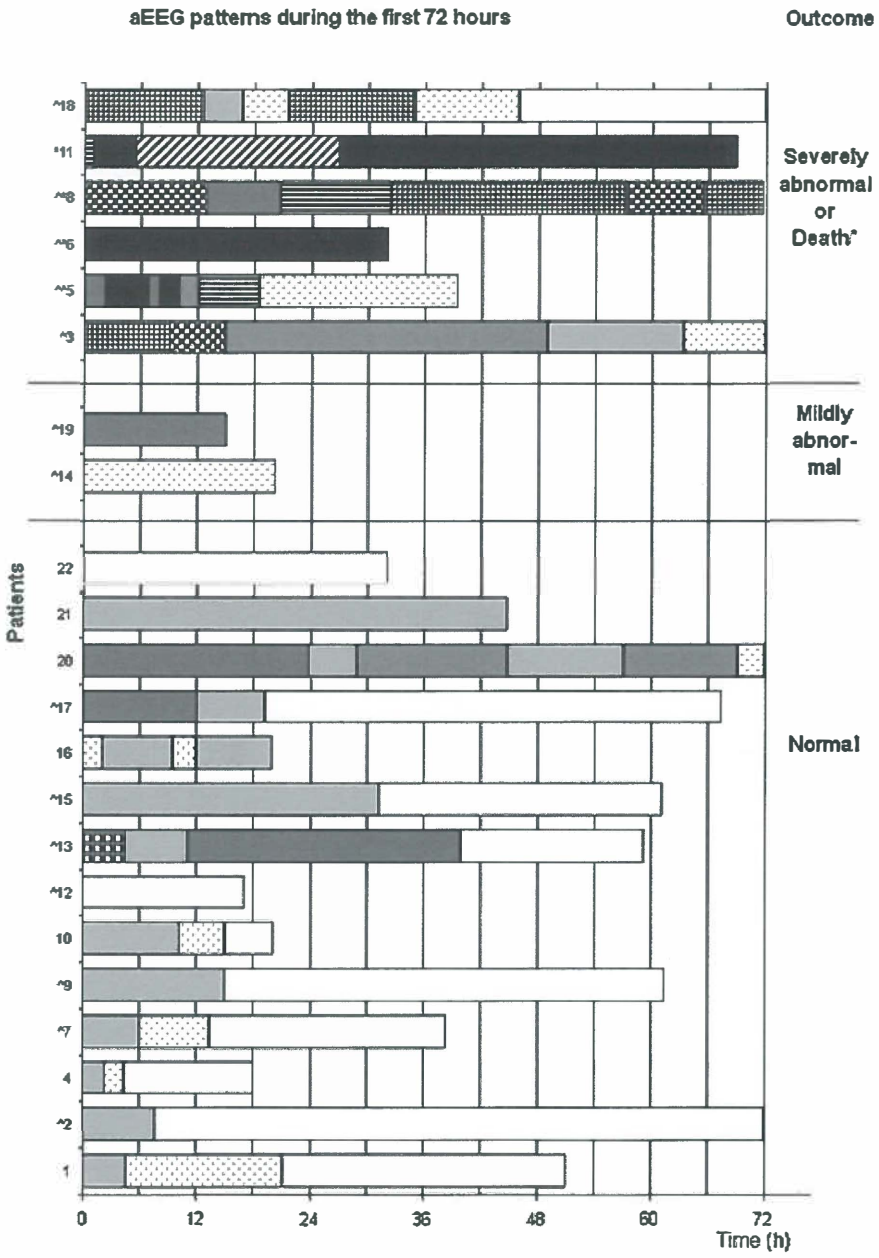

Figure 1: Longitudinal course of aEEG during the first 72 hours after admission.
*: died, ^: meningitis.

CNV + SWC: ; CNV: ;DNV: ;BS: ;CLV: ;FT: ;
SE: ;CNV/DNV +EA: ;BS +EA: ;FT + EA:

count between infants with a normal and an adverse outcome. C-reactive protein was significantly higher in infants with an adverse outcome (Mann-Whitney U, $p < 0.05$).

Four infants (18%) died of which three had meningitis. The survivors were seen for follow-up at our outpatient clinic at regular intervals. Follow-up consisted of both a paediatric and neurological examination. The paediatrician performing the follow-up did not know the results of the aEEG evaluation. Neurological findings were classified as normal, mildly abnormal (MA: neurological abnormalities other than cerebral palsy or infantile spasms, e.g. co-ordination problems, hearing loss) or severely abnormal (SA: severe mental and/or motor delay, infantile spasms or cerebral palsy). Of the surviving 18 infants follow-up results were available for all infants at the age of 24 months. Two Infants (11%) were severely abnormal at follow-up. Two infants were mildly abnormal and 14 infants were normal at follow-up.

Background pattern

In nine infants there was a low voltage background pattern (FT, CLV or BS) at some stage in the first 72 hours after admission. In these nine infants, BS was the most commonly recorded abnormal background pattern (5 infants), CLV was seen in one infant and FT was seen in three infants. In five of the nine infants the background pattern improved to DNV or CNV during the first 72 hours and in two within 24 hours. All infants with CLV or FT had an adverse outcome. Of the remaining 13 infants, 12 had CNV or DNV without EA throughout their recording. Eight of them started with DNV, of which seven improved to CNV. Three infants had CNV and one had DNV for the duration of the entire recording. In one infant CNV changed to DNV. The aEEG background patterns related to outcome (Table 2).

All infants with CNV or DNV without EA for the duration of the entire recording or re-

Table 2: Relation between aEEG patterns and neurological outcome

		Neurological outcome at 24 months				
		Normal	MA	SA	Dead	Total
Worst aEEG pattern	CNV	2	1			3
	DNV	9				9
	BS	3	1	1		5
	CLV or FT				4	4
	SE			1		1
Total		14	2	2	4	22

Chi ^{2} for trend, $p < 0.001$. SA: severely abnormal; MA: mildly abnormal; CNV: continuous normal voltage; DNV: discontinuous normal voltage; BS: burst suppression; CLV: continuous low voltage; FT: flat trace; SE: status epilepticus .

Table 3: Positive likelihood ratio (LR+) of low voltage aEEG patterns (BS,CLV or FT) and electrographic seizure activity (EA or SE) for adverse outcome (death or severely abnormal at 24 months).

Time after admission	LR+	95%CI	N
Low voltage patterns			
at 6 h	5.3	1.9-14.8	20
at 12 h	5.3	1.9-14.8	21
at 24 h	8.3	1.3-55	16
at 48 h	> 4.7		10
EA/SE any time during recording	10.6	1.5-76	22

BS: burst suppression;CLV: continuous low voltage; FT: flat trace; EA: electrographic epileptic activity; N: number of infants

covery to CNV or DNV within 24 hours had a normal outcome or MNA (chi-squared for trend; $p < 0.001$). Low voltage patterns (BS, CLV or FT) had a positive likelihood ratio (LR+) for an adverse outcome that was the highest at 24 hours after admission (Table 3).

The clinical condition of infants with low voltage background patterns did not differ from infants with DNV or CNV patterns, with respect to gestational age, the need for inotropes, ventilation, clinical seizures, use of AED, lactic acidosis, neurological condition, etc.

Electrographic seizure activity

Five infants had electrographic seizure activity (EA) in their recordings. Three had repeated seizures and two had SE. In four cases, there were also clinical seizures. There were an additional eight infants who had clinical seizures without evidence for EA on aEEG. Seven of these eight infants were already treated with AED prior to the start of aEEG recording. Clinical seizures were as frequent in infants with a normal outcome as in infants who died or had an adverse outcome. EA was significantly more frequent in infants with an adverse outcome than in infants with a normal outcome (Fisher's Exact, $p < 0.01$). EA at any time during the recording had a LR + of 10.6 (95% CI 1.5-76) and a negative likelihood ratio of 0.35 (95% CI 0.1-1.1) for an adverse outcome (Table 3).

All infants with clinical seizures (n =12) were treated with AED according to our protocol. Drug of first choice was phenobarbitone with a maximum loading dose of 30 mg/kg. In case of persistent seizures midazolam was started (loading dose 0.05 mg/kg, maintenance dose 0.15-0.2 mg/kg/h). If control of seizures was still not

achieved, lidocaïne was added (loading dose 2 mg/kg, maintenance dose 6 mg/kg/h, reduced at 6 and 18 h and stopped at 30 h). After the treatment with phenbarbitone there was a temporary change with a more depressed background pattern in two of the infants. The remaining ten infants did not show any change in background pattern. Regarding midazolam treatment, BS appeared in two of the five infants treated during aEEG recording, following successful treatment of SE. Three infants had FT or CLV. This existed already before midazolam treatment in two infants and followed BS in one infant.

Sleep wake cycling

Sleep wake cycling was present in 13 of the 22 infants. If present, cycling commenced at a mean time of 17 h after admission. SWC appeared significantly more frequently in infants with a normal outcome (Fisher's Exact, $p < 0.05$). In the infants with a normal outcome or mild neurological impairment cycling appeared in 12 of 16 infants. In the six infants with an adverse outcome it appeared only in one.

Differences in aEEG between patients with sepsis or meningitis

Low voltage background patterns appeared as frequently in infants with sepsis as in infants with meningitis. Clinical seizures were significantly more frequent in infants with meningitis (Fisher's Exact, $p < 0.05$), but there was no difference in EA between the two groups. The same was true for the appearance of SWC. There was also no difference in the time of onset of SWC.

Neuro-imaging

Of the 22 infants 7 had abnormal cerebral ultrasound results (Table1). Of these 7 infants 6 had adverse outcomes (chi-squared for trend; $p < 0.001$). Ultrasound data related significantly to aEEG data (chi-squared for trend; $p = 0.003$). All infants with normal ultrasound combined with normal aEEG had fair outcomes, where infants with abnormal ultrasound combined with severely abnormal aEEG all had adverse outcomes. The four infants with severely abnormal aEEG (all BS) that had normal cerebral ultrasound had fair outcomes. One infant with an abnormal ultrasound had a normal aEEG. This particular infant had a fair outcome.

Discussion

The present study indicates that severely abnormal aEEG background patterns are not uncommon in infants with a neonatal sepsis or meningitis. Low voltage patterns within the first 48 hours after admission are predictive for a poor neurological outcome or death. Studies that used conventional EEG in infants with bacterial meningitis reported similar findings (4, 14, 15). In some infants a low voltage background on aEEG improves to become a more continuous pattern, which is associated with a relatively good outcome. aEEG has an obvious advantage over EEG. Continuous monitoring makes it possible to observe both improvement and worsening of the background pattern over time, where conventional EEG in general is recorded intermittently and for a short time-period. In our opinion continuous neurological monitoring is of importance in at risk infants, especially in infants where clinical

assessment is difficult, due to ventilation, use of sedatives etc. Only one infant was neurologically normal during the aEEG recording. The clinical assessment was largely influenced by the use of medication (AED, morphine, neuromuscular blocking agents). We found no differences in the clinical condition with respect to clinical and electrographic seizures, neurological condition, use of sedatives or AED, ventilation, use of inotropes, and lactic acidosis at admission between infants with severely abnormal aEEG patterns (FT, CLV, BS) and infants with normal (CNV ± SWC) or moderately abnormal aEEG patterns (DNV).

Our findings on aEEG in this patient population are similar to studies involving infants with perinatal asphyxia with respect to the relation between outcome and low voltage background patterns (6, 7). This is also true for the recovery of low voltage background patterns over time. Normalisation of the aEEG background pattern following asphyxia is also associated with a relatively good outcome (7, 16).

Clinical seizures occurred frequently in this study cohort, more than 50% of infants had clinical seizures. The high incidence of clinical seizures might be explained by the fact that all these infants were admitted to a level III NICU and are therefore proportionally the most severely ill group of infants suffering from neonatal sepsis and/or meningitis. In 60% of the infants with clinical seizures there was no EA visible on aEEG. It is known that brief seizures and focal seizures are not detected by aEEG (8). It can also be a reflection of the successful treatment of seizures because a majority of these infants was already treated with AED before aEEG monitoring was started. Another explanation might be that diagnosing clinical seizures remains very difficult; a study using continuous video-EEG monitoring showed that only 27% of suspected clinical seizures had corresponding electrographic manifestations (17). We found that seizure activity on aEEG was highly predictive for adverse outcome, and not clinical seizures. This is a new finding and it emphasizes the need for continuous (a)EEG monitoring for longer periods. Our data is comparable with previous EEG data that showed an association between electrographic seizures and mortality and morbidity in at risk infants (18). To our knowledge, there is no NICU with round-the-clock EEG monitoring with on-line assessment available. Therefore, a CFM is the best mode of continuous monitoring at this moment. Furthermore, with the use of digital equipment, one is able to record a single lead EEG using the already present electrodes, thus aiding the proper interpretation of the aEEG (11, 12).The immature brain is susceptible to neuronal damage due to seizures (19, 20). Although it remains controversial as to whether subclinical seizures should be treated (21), aEEG monitoring makes it possible to treat electrographic seizures or SE immediately. In this way, one might protect the brain from additional damage.

SWC appeared more frequently in infants with a fair outcome. This is in line with previous research addressing the appearance of SWC following perinatal asphyxia. Earlier onset of SWC following asphyxia is associated with a favourable outcome (22). We therefore recommend continuing the recording of aEEG in any infant with encephalopathy until SWC appears.

Several factors might be responsible for the changes in aEEG pattern in infants with sepsis and or meningitis. Diminished cerebral perfusion due to circulatory insufficiency might depress electro-cortical activity (23). However we did not find any significant differences in the blood pressure, nor in the need for inotrops between the groups. Another factor might be the changes in cerebral microcirculation in response to inflammation (24, 25). Depression of the aEEG might also be caused by AED, midazolam in particular (26, 27). However, in only two infants we did observe changes in background pattern after the administration of phenobarbitone. Following midazolam infusion a change of background pattern from DNV or CNV to BS can be observed. A change to traces such as CLV or FT has not been reported (27). In this study BS was only present after the successful treatment of SE. CLV or FT was present in three infants. In two of these infants this was already present before treatment with midazolam. In this group, it seems that midazolam had no major influence on aEEG.

At lower gestational ages the background pattern of aEEG is more profoundly discontinuous (28). Six infants were born between 34 and 37 weeks of gestation. At a gestational age above 34 weeks background pattern in general is continuous with presence of SWC (29,30). Only one preterm infant had a severely abnormal background pattern (FT) during the entire recording. This particular infant died. The remaining five near term infants all had a CNV or DNV pattern, none had a BS pattern. There was no difference in the gestational age between infants with severely abnormal aEEG patterns (FT, CLV, BS) and infants with normal (CNV ± SWC) or moderately abnormal aEEG patterns (DNV). This indicates that aEEG is also helpful in near-term infants with sepsis or meningitis.

There are some limitations of the study. There is a relatively small sample size, but to our knowledge this is the first study that reports on the use of aEEG in infants with neonatal sepsis/meningitis. The study population is heterogeneous, but infants are comparable with respect to the severity of illness. The study is retrospective and therefore the length of aEEG recordings is not equal in all infants. On the other hand aEEG recordings were generally only discontinued if aEEG was normal or had normalised. Conventional EEG was not performed routinely when aEEG was being recorded. We do not regard this as a major limitation as it has been shown that aEEG recordings are in good agreement with conventional EEG recordings (8, 9).

Conclusions

The present study indicates that severely abnormal background patterns (FT, CLV and BS) and electrographic seizure activity on aEEG are not uncommon in infants with neonatal sepsis and/or meningitis. Sleep wake cycling (SWC) appears more frequently in infants with a good outcome. In infants with neonatal sepsis and/or meningitis continuous aEEG monitoring for longer periods is an adjunct to clinical observation and can be helpful in predicting neurological outcome.

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PART 3

Pitfalls in amplitude integrated EEG recording

CHAPTER 7

Burst suppression on amplitude integrated EEG may be induced by midazolam. A report of three cases.

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Abstract

Continuous amplitude integrated EEG (aEEG) recording with a cerebral function monitor is a useful tool to evaluate prognoses following perinatal asphyxia in term infants. Drugs may change the pattern of the conventional EEG. This report presents three infants treated with midazolam because of status epilepticus and repetitive seizures who proved resistant to other anti-convulsants (phenobarbitone, lidocaine). The infants developed burst suppression patterns on aEEG concurrent with high serum levels of midazolam (900-7093 µg/l). Following discontinuation of midazolam treatment, serum levels normalised and background patterns returned to normal voltage traces.

Conclusion: these findings indicated that midazolam can cause burst suppression on aEEG. Therefore, the prognostic value of aEEG is limited in case of high serum levels of midazolam. Serum levels of midazolam should be measured in infants who have burst suppression patterns on aEEG during midazolam treatment.

Introduction

Amplitude-integrated EEG (aEEG) recorded with a cerebral function monitor is an accurate method for assessing brain function in term infants with encephalopathy, e.g. in case of severe perinatal asphyxia (1). The aEEG records a single channel EEG from biparietal electrodes; frequencies < 2Hz and >15Hz are filtered selectively, and the amplitude of the signal is integrated. The processed signal is recorded semi-logarithmically onto a printer with a low paper speed (6 cm/h) (2). Previous studies have shown that aEEG correlates well with conventional EEG (3). Within hours following perinatal asphyxia, aEEG has a prognostic accuracy of 80-85% for neurological outcome (4, 5). Burst suppression patterns at 3 and 6 hours post partum in term neonates following asphyxia are associated with poor neurological outcome (6). At this early age, aEEG recording is the most suitable method to identify infants eligible for trials investigating neuroprotective treatment following asphyxia. Besides its accuracy in predicting neurological outcome, aEEG can also help to identify (subclinical) seizures (7).

Because aEEG is used to evaluate prognoses, it is important to know the influence of certain frequently used drugs on the trace. Phenobarbitone may change the background pattern of the aEEG for a short period, but this is a rare condition in term infants with mild encephalopathy (8). In preterm infants phenobarbitone and morphine can cause depression of the background pattern of the aEEG (9). During continuous intravenous treatment with midazolam in children suffering refractory status epilepticus, burst suppression may develop on EEG (10, 11). In critically ill adult patients, midazolam changes the spectrum of frequencies and the logarithm of power of the EEG (12).

In the authors' unit, a level III neonatal intensive care unit (NICU), aEEG recording is routinely performed in all patients following perinatal asphyxia or encephalopathy of unknown origin and if seizures are suspected. Approximately 40 term infants meeting these criteria are admitted each year. Recordings are initiated immediately after admission, using the Lectromed® Multitrace 2, and usually last for several days, until abnormalities have resolved or are unlikely to recur. The aEEG traces are interpreted by the attending neonatologist, and categorised into several patterns, according to Toet et al. (6): continuous normal voltage, discontinuous normal voltage, burst suppression, low voltage traces and flat trace. Status epilepticus and repetitive epileptic discharges are also identified. In the unit, infants with seizures, recognised clinically or electrographically on aEEG as status epilepticus or repetitive seizures, are treated according to protocol. The drug of first choice is phenobarbitone with a maximum loading dose of 30 mg/kg. In cases of persistent seizures lidocaine is started (loading dose 2 mg/kg, maintenance dose 4-6 mg/kg/h, reduced after 24 h and stopped at 48 h). If control of epileptic activity has still not been achieved, midazolam is added.

This study presents three infants suffering seizures treated with midazolam as the drug of third choice. In all patients burst suppression occurred on aEEG. The implications of these findings are discussed.

Case 1

The first case is a boy, born in a local hospital at a gestational age of 39 wk and 2 d. His birth weight was 3950 g. His mother's pregnancy was complicated by maternal insulin-dependent diabetes mellitus. Delivery was induced by oxytocine, because of high diastolic blood pressure. Labour did not progress and a Caesarean section was performed. Apgar scores were 2 and 4 after 1 and 5 min, respectively. Because of respiratory failure, the infant was intubated and artificially ventilated. Umbilical arterial pH was 6.91. After resuscitation sodium bicarbonate was administered and the infant was transferred to the NICU. On admission, background pattern on aEEG was mildly abnormal, showing discontinuous normal voltage. Within a few hours the boy developed clinical seizures, with repetitive epileptic activity on aEEG. Bloodanalysis did not reveal a cause for the seizures: electrolytes, glucose and acid-base balance were normal. Ultrasound of the brain revealed a small subependymal haemorrhage. Besides signs of postanoxic encephalopathy the infant had multiorgan failure with elevated liver enzymes and oliguria. The seizures were attributed to hypoxic-ischaemic encephalopathy and treated with phenobarbitone (30 mg/kg, maintenance dose 5 mg/kg/d). Because of persistent seizures lidocaine was added (loading dose 2 mg/kg in 10 minutes, maintenance dose 6 mg/kg/h). The infant continued to have seizures and was put on midazolam 32 h after birth. After a loading dose of 0.05 mg/kg in 5 min, a maintenance dose was started at 0.2 mg/kg/h, and gradually increased to a dose of 0,9 mg/kg/h within the next 36 h. Fifteen minutes after midazolam therapy was initiated, at a dose of 0.2 mg/kg/h, aEEG changed to burst suppression. A blood sample 54 h later, on the third day, revealed a serum level of

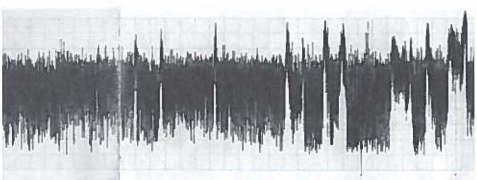

Figure 1a: Discontinuous normal voltage with epileptic discharges in patient 1

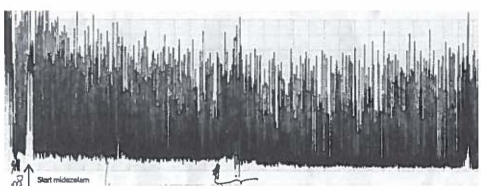

Figure 1b: Burst suppression after start of midazolam in patient 1

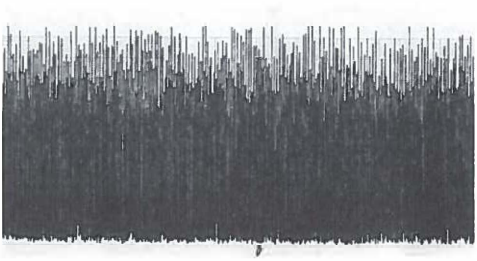

Figure 1c: Burst suppression during continuous treatment with midazolam in patient 1

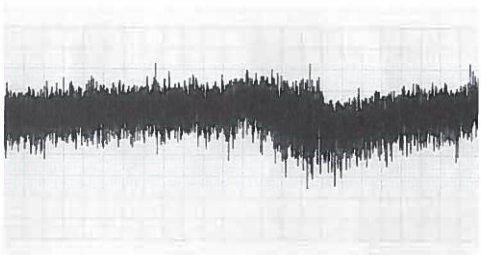

Figure 1d: Continuous normal voltage after withdrawal of midazolam in patient 1

midazolam of 7093 µg/l (normal = 80-250 µg/l). Serum level of phenobarbitone at that time was 39 mg/l (normal = 20-40 mg/l). Because of the high serum level, midazolam was withdrawn. Midazolam serum levels gradually declined to 180 µg/l within 4 d. Concurrently, aEEG changed from burst suppression to continuous normal voltage, without signs of epileptic activity anymore. AEEG patterns of this patient are shown in Figure 1a-d. A magnetic resonance imaging (MRI) scan of the brain at the end of the first week of life revealed no abnormalities. The infant was extubated 8 d after birth and was transferred to a local hospital. He is seen regularly for follow-up in the outpatient clinic of the University Hospital groningen. At the age of 6 mo he has mild tonus regulation abnormalities and strabismus.

Case 2

The second case is a boy born at home. Pregnancy and delivery were without complications. Nonetheless the boy was born in poor condition: pale, flaccid and suffering from bradycardia. The midwife started resuscitation and within 5 min after birth heart rate was above 100/minute. At arrival in a local hospital, 1 h after delivery, the infant suffered respiratory and circulatory failure. He was intubated and artificially ventilated. Because of hypotension he received fresh frozen plasma. Bloodanalysis revealed anaemia (Hb 5,2 mmol/l) and red blood cells were transfused. The anaemia remained unexplained; there were no fetal cells in the maternal circulation, nor were there signs of hemolysis. The infant was transferred to our NICU for intensive care treatment. At admission, aEEG revealed a pattern of discontinuous normal voltage. About 24 hours after birth the infant developed clinical seizures, also identified on aEEG. Treatment was started with phenobarbitone (loading dose 30 mg/kg, serum level 17 mg/l), lidocaine and ultimately midazolam. Besides neurological signs of hypoxic-ischaemic encephalopathy the infant developed multi-organ-failure. Midazolam was started (loading dose 0.1 mg/kg, maintenance dose 0.1 mg/kg/h, gradually increased within 3 h to a maximum dose of 0.7 mg/kg/h), after which seizures disappeared. Within 30 min after starting midazolam therapy, a burst suppression pattern developed on aEEG. Conventional EEG confirmed burst suppression. A bloodsample 12 hours after the start of midazolam revealed a serum level of midazolam of 3960 µg/l. Subsequently, midazolam was withdrawn, and serum levels of midazolam gradually declined to 500 µg/l. Concurrently, aEEG changed to discontinuous normal voltage. Neuro-imaging (repetitive brain ultrasound scans) revealed slight oedema of the brain, that disappeared within the first week of life. The infant was artificially ventilated for 3 wk, also due to oedema caused by renal insufficiency for which peritoneal dialysis was started. Ultimately renal and hepatic function did not improve and the boy died at the age of 3 mo from renal and liver insufficiency.

Case 3

The third case was a boy, born in this hospital at a gestational age of 38 wk and 5 d. His birthweight was 4450 grams (>P90). He was the firstborn of a healthy mother. Apgar scores were 8 and 10 after 1 and 5 min, respectively. Shortly after birth the child developed severe and prolonged hypoglycaemia (0.1 mmol/l) and was admitted to the

NICU. A glucose-intake of 14 mg/kg/min was needed to reach adequate glucose concentrations. Diagnostic tests did not reveal any cause (inborn error of metabolism, endocrinologic disorder). Several hours after the hypoglycaemic period, the infant developed clinical seizures. By that time serum glucose had become normal. No cause for the encephalopathy was identified other than neuronal compromise after prolonged and severe hypoglycaemia. The aEEG recording revealed status epilepticus. The seizures were treated with phenobarbitone (serum level 30 mg/l), lidocaine and midazolam (loading dose of 0.05 mg/kg, maintenance dose of 0.1 mg/kg/h, gradually increased to a maximum dose of 0.4 mg/kg/hr). A few hours after midazolam was initiated the status epilepticus on aEEG changed to burst suppression. Twelve hours after midazolam had been started the serum level was 900 µg/l. Midazolam was withdrawn and aEEG gradually changed to continuous normal voltage, and the serum level of midazolam dropped below 100 µg/l. Neuro-imaging revealed a small subependymal haemorrhage. MRI at the age of one month did not reveal any hypoxic-ischaemic related abnormalities. Although there were no signs of multi-organ-failure, the infant was oliguric in the first 2 d of life. He was discharged home after 4 wk and is regularly seen for follow-up in the outpatient clinic. He displayed a mild axial hypotonia at the age of 3 mo.

Discussion

This study reported on three infants treated with midazolam for status epilepticus and repetitive seizures. During treatment burst suppression patterns developed on aEEG and this coincided with high serum levels of midazolam.

Continuous intravenous midazolam is an effective treatment for status epilepticus in children (10, 11). Igartua et al. reported about the treatment of refractory status epilepticus with midazolam coma, using 0.25-1.45 mg/kg/h (10). In the present study, midazolam treatment with doses ranging from 0.4 to 0.9 mg/kg/hr effectively terminated seizure activity, both clinically and on aEEG recordings. However, this treatment resulted in high serum levels (900-7093 µg/l), which in turn resulted in a burst suppression patterns on aEEG. In all 3 cases, burst suppression patterns disappeared after serum levels of midazolam had normalised, and aEEG regained the same background pattern as before midazolam therapy. In two infants burst suppression occurred soon after the loading dose. It is possible that after a single loading dose of midazolam, the background pattern of the aEEG may already change into a burst suppression pattern, which persists during maintenance therapy with gradually increasing serum levels during the next hours and days. Because serial blood samples were not taken during the initial phase of midazolam treatment, it was not possible to determine at which serum level of midazolam burst suppression developed. Treatment with barbiturates may also have contributed to the effect of midazolam. However, changes in background pattern due to other anti-epileptic drugs are short lasting and uncommon in infants with mild encephalopathy (2, 8). Since background patterns on aEEG were normal after introducing barbiturates and serum levels of phenobarbitone were always within normal range, it is unlikely that barbiturate treatment by itself generated the burst suppression patterns.

Serum levels of lidocaine were not taken. Lidocaine was administered according to the protocol described by Hellström-Westas et al. (13). After a loading dose of 2 mg/kg, a maintenance dose of 4 mg/kg/h was given. If seizures persisted, the dosage was increased to 6 mg/kg/h. Lidocaine dosage is diminished after 24 h and stopped after 48 h. Adverse effects of lidocaine and its metabolites are not seen if given for a short period like this and, therefore, measuring serum levels has no clinical value (14). Depression of the background pattern on aEEG has been reported, but is mostly transient (13). In our patients no changes of aEEG trace were observed after introducing lidocaine.

It could be argued that the change of discontinuous normal voltage into burst suppression is due to the natural course of aEEG patterns following hypoxic-ischaemic or hypoglycaemic encephalopathy. The authors do not think this to be is the case. According to animal experiments, EEG intensity is decreased for several hours following perinatal asphyxia (15). Burst suppression and slightly later seizure activity accompanies the secondary phase of neuronal injury. The same holds true for human studies on asphyxiated infants (6). In the current study, all infants had discontinuous normal voltage background patterns (only mildly abnormal traces) and not burst suppression before midazolam treatment. In addition, neuro-imaging did not reveal obvious signs of hypoxic-ischaemic neuronal damage.

Midazolam is metabolised by the cytochrome P450 enzyme system. More than 90% of midazolam and its metabolites is cleared by the kidney (16). Metabolism and clearance rates are reduced considerably in critically ill newborns compared with older children and adults (17). Moreover, the infants presented here suffered multi-organ-failure or oliguria. All of these factors may have contributed to the high midazolam levels. In the authors' opinion, midazolam dosage should be increased with great caution and doses above 0.2 mg/kg/h should be avoided in critically ill newborn infants.

Amplitude-integrated EEG is a reliable method to predict neurological outcome after perinatal asphyxia in term infants, with an accuracy of 80-85% at 6 h after birth (6). Burst suppression after perinatal asphyxia is associated with poor neurological outcome. The question is whether drug-induced burst suppression (e.g. midazolam) on aEEG is associated with poor neurological outcome. Even though only three cases were described here, the findings indicated that this might not be the case. Therefore, assessment of neurological outcome based on the aEEG is unreliable as long as serumlevels are high. Serum levels should be checked in case of burst suppression during midazolam therapy. If levels are low, burst suppression may be caused by asphyxia. If levels are high burst suppression might be drug induced.

Conclusions

Midazolam is a potent drug to treat status epilepticus and repetitive seizures in term newborns with encephalopathy. However, the data indicated that midazolam in high doses might lead to high serum levels and to burst suppression on aEEG, which is

of limited prognostic value under these circumstances. To avoid high serum levels of midazolam, the authors recommend increasing the dose of midazolam to a maximum of 0.2 mg/kg/hr. If higher doses are deemed necessary, therapy should be monitored with serum levels. Serum levels should also be checked in case of burst suppression on aEEG.

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CHAPTER 8

The added value of simultaneous EEG and amplitude-integrated EEG recordings in three newborn infants

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Abstract

Amplitude-integrated electroencephalograms (aEEGs) recorded by cerebral function monitors (CFMs) are used increasingly to monitor the cerebral activity of newborn infants with encephalopathy. Recently, new CFM devices became available which also reveal the original EEG signals from the same leads. To date it was unclear whether this single-lead EEG provides additional information towards interpreting the aEEG traces more accurately. Our report deals with three cases in which the single-lead EEG from the CFM device did indeed reveal important additional information not provided by the aEEG alone. In cases 1 and 3 the aEEGs showed drifting of the baseline to higher amplitudes. The single-lead EEG revealed that this was due to muscle artefacts, high-frequency oscillation ventilation (HFOV) and the electrocardiogram rather than to cerebral activity. Hence, without knowledge of the EEG, the aEEG trace might have been misinterpreted as being fairly normal. Case 2 showed paroxysmal elevation of the lower margin of the amplitude on the aEEG which looked like epileptic activity. However, additional information from the single-lead EEG revealed that it was due to muscle artefacts. Thus, simultaneously recorded EEG can help to interpret seizure-like episodes on the aEEG. Conclusion: Simultaneously recorded single-lead EEGs can help to interpret aEEG traces more accurately.

Introduction

The amplitude-integrated electroencephalograms (aEEGs) recorded by cerebral function monitors (CFMs) are used increasingly in neonatal intensive care. It is an accurate method to assess brain function in term infants with encephalopathy (1-3). The aEEG is a continuous, on-line recording of cerebral electrical activity at the cot-side. The CFM records a single-lead EEG from biparietal electrodes. The signal is filtered in order to reduce frequencies below 2 Hz and above 15 Hz. The amplitude is integrated before it is written out at slow speed (6 cm/h). The background pattern is assessed by pattern recognition. Because of the long periods of continuous registration the CFM is especially useful to evaluate changes in background patterns over time and to detect seizures. Accordance between EEG and aEEG is high (1, 4, 5).

Although there are some pitfalls in interpreting background patterns on the aEEG traces, only a few reports about these pitfalls are available (6). It has been reported that certain focal, low-amplitude and very short periods of seizure discharges can be missed on aEEG traces (4). Furthermore, it can be difficult to distinguish between seizures and artefacts on the aEEG, e.g. due to handling of the infant. A standard EEG can be helpful in these situations (7), but it is only a momentary recording and usually not directly available when difficult and doubtful aEEG traces are being interpreted.

In our level III neonatal intensive care unit, aEEG recordings are routinely performed in all patients with encephalopathy following either perinatal asphyxia or any other origin, and if seizures are suspected. The attending neonatologist interprets the aEEG traces which have been categorised into several patterns according to Toet et al. (8): continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage (CLV) and flat trace (FT). In addition, the presence of status epilepticus and repetitive epileptic discharges are identified. Until recently recordings were made using the Lectromed® Multitrace 2. Since September 2003 we are also using a new digital device (Olympic® CFM 6000) which displays both the aEEG and the original single-lead EEG. Using this device we found that in a substantial number of cases the single-lead EEG provided additional information which proved important for the accurate interpretation of the aEEG recordings. In this article we report on three such cases that represented the typical pitfalls in interpreting aEEG traces.

Case 1

The first case was a boy born at our hospital at a gestational age of 29 weeks. His birth weight was 2030 g. The Apgar scores were 1, 5 and 6 after 1, 5 and 10 min. The infant suffered from respiratory distress, sepsis, asphyxia and pulmonary hypertension. Because of increasing respiratory failure the ventilation mode was changed from synchronised intermittent positive pressure ventilation to HFOV. Because of birth asphyxia aEEG was recorded (Figure 1a, b).

The background pattern of the aEEG trace initially showed a pattern with the lower

margin of the amplitude around 8 μV with a high burst-density (Figure 1a). Considering the lower margin, this could be interpreted as a DNV background pattern. However, the single-lead EEG, recorded at that time (arrow), showed BS. During the period of suppression, oscillations of 10 Hz caused by the HFO ventilation were visible on the EEG trace. About one hour later, without having handled the infant, the background changed to a lower voltage with a broader bandwidth (Figure 1b). Again, this could be interpreted as DNV, but now with a lower baseline, and still with a high burst-density. The single-lead EEG recorded at that time (arrow) still showed BS, but the oscillations were less clear.

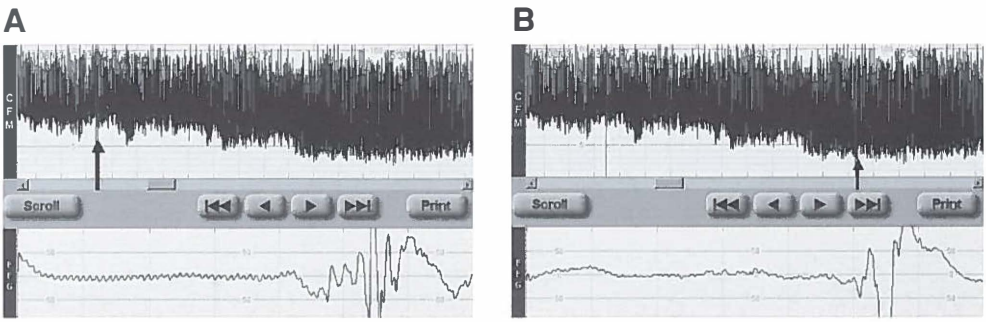

Figure 1: aEEG patterns and single-lead EEG in case 1. The upper part of the graph shows the aEEG during approximately three hours. The lower part of the graph shows the single-lead EEG at the moment of the arrow, during 7 seconds.
(a) Arrow: aEEG shows a normal voltage background pattern. Single-lead EEG shows BS, with oscillations due to HFO ventilation during suppression.
(b) Arrow: One hour later the background pattern changes to a more discontinuous pattern. The single-lead EEG still shows BS, but the oscillations are less clear.

Clinically the patient made a rapid recovery. The aEEG changed to BS with shorter interburst intervals on the single-lead EEG. No standard EEG was performed. An ultrasound scan of the brain revealed a germinal matrix haemorrhage on the left side. The patient was extubated on the third day of life and could be discharged on the thirteenth day of life to a local hospital. Follow-up at twelve months postterm showed a normal neurodevelopment outcome.

Case 2

The second case was a girl born at our hospital at a gestational age of 33-34 weeks. Her birth weight was 3200 g. Her mother was diethylstilbestrol-exposed in utero. Pregnancy was induced by in vitro fertilisation. Because of macrosomia, the mother was tested for glucose intolerance, which did not reveal any abnormalities. During delivery the foetal cardiotocogram and scalp blood gases did not show any abnormalities. The Apgar scores were 7, 8 and 8 after 1, 5 and 10 min. She suffered from

mild respiratory distress syndrome for which she was ventilated during several hours. One hour postpartum she had a hypoglycemia of 1.1 mmol/l, for which she received an intravenous bolus of glucose. Serum glucose levels remained normal afterwards. Because she developed clinical seizures within 24 h after birth, aEEG was recorded (Figure 2a, b).

Figure 2a shows elevations of the lower margin of the amplitude on aEEG that could be interpreted as seizures. The single-lead EEG recorded at the time of the largest elevation (arrow) indeed showed epileptic activity superimposed on a trace resembling an electrocardiogram (ECG). The seizures were treated with phenobarbitone. About 12 h later the background again showed elevations of the lower margin of the amplitude (Figure 2b). This time, the single-lead EEG (arrow) showed a high frequency pattern reflecting muscle activity. Standard EEG performed on the third day of life showed a normal background pattern without epileptic activity which corresponded to the infant's postmenstrual age.

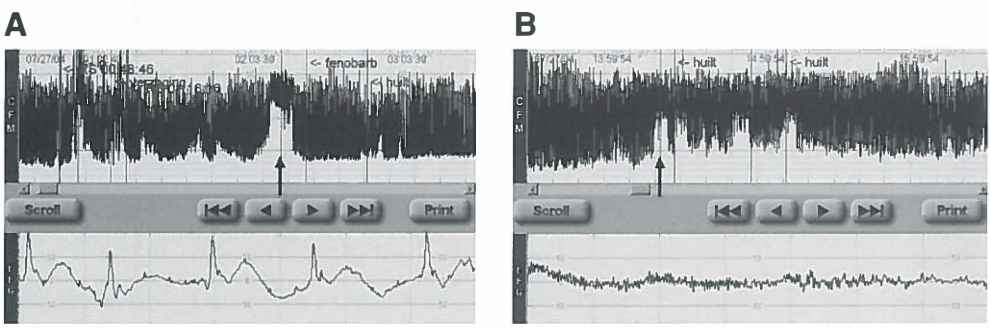

Figure 2: aEEG patterns and single-lead EEG in case 2. The electrode impedance (not shown in the graph) was normal throughout the recording.

(a) Arrow: The elevations of the lower margin of the amplitude on the aEEG can be interpreted as seizures. The single-lead EEG reveals epileptic activity with an ECG in between.

(b) Arrow: About twelve hours later, and after treatment with phenobarbitone, the background pattern on the aEEG trace again shows elevations of the lower margin of the amplitude. The single-lead EEG does not reveal epileptic activity but a high frequency pattern which reflects muscle activity.

This patient's cerebral ultrasound scan eventually showed periventricular leucomalacia grade 3. This was confirmed by magnetic resonance imaging on day 12 which revealed multiple periventricular and paraventricular cysts. Magnetic resonance spectroscopy showed elevated lactate levels and decreased N-acetyl aspertate levels. At 10 months postterm the girl had a neurodevelopmental delay of 4 months and she had developed spastic quadriplegia.

Case 3

The third case was a boy born at term at a local hospital. His birth weight was 4440 g. Labour was complicated by meconium stained amniotic fluid and acidosis (foetal scalp blood pH 6.91, BE –16). The Apgar scores were 0 and 0 after 1 and 5 min.The infant was resuscitated. After 20 minutes he started to gasp and he had a spontaneous heart rate of ≥ 100/min. After a short period of stabilisation, the boy was transported to our hospital for further evaluation and treatment. Apparently, he had suffered from severe perinatal asphyxia caused by prolonged labour. On admission, it appeared that he suffered from encephalopathy stage 3 according to Sarnat, he was comatose and made myoclonic movements. Because of the hypoxic-ischemic encephalopathy aEEG recording was initiated (Figure 3a, b).

At first the aEEG showed a pattern with the lower margin of the amplitude around 8 μV (Figure 3a). This could be interpreted as a CNV pattern. However, the single-lead EEG at that time (arrow) revealed muscle artefacts and an electrocardiogram. As the boy was artificially ventilated, the muscle relaxant vecuronium was administered in order to obtain an accurate aEEG without artefacts. The aEEG trace after vecuronium (Figure 3b) changed to discontinuous pattern with the lower margin of the amplitude around 5 μV. The single-lead EEG recorded at that time (arrow) showed an iso-electric trace with an ECG. A conventional EEG performed during the latter period showed BS with long periods of iso-electricity and no differentiation or reactivity. Activity in the centroparietal parts of the brain was absent during bursts.

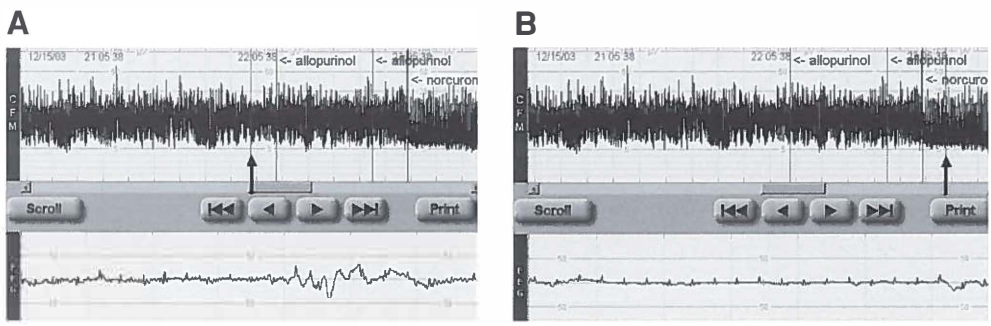

Figure 3: aEEG patterns and single-lead EEG in case 3.
(a) Arrow: The aEEG shows a CNV pattern. The single-lead EEG reveals a high frequency pattern, which reflects muscle activity.
(b) Arrow: aEEG after vecuronium shows a discontinuous pattern. However, the single-lead EEG reveals an iso-electric trace with an ECG.

After several days, during which the clinical condition of the boy showed no signs of improvement and the EEG remained nearly iso-electric, intensive care treatment was withdrawn because of severe hypoxic-ischaemic encephalopathy (Sarnat 3). The patient died after ventilation was ceased.

Discussion

aEEG is an easy and helpful instrument for round-the-clock, cot-side cerebral monitoring. This report clearly demonstrates the added value of the simultaneously recorded original EEG, in combination with the possibility of reviewing aEEG traces in retrospect. It allows us to identify baseline drift (e.g. due to ECG and HFOV or to muscle activity) and to distinguish between artefacts, muscle activity and seizures. Reading the original EEG trace in these instances does not require much additional training for neonatologists. Regular patterns such as the ECG, the 10Hz oscillations and the repetitive high voltage discharges seen in epileptic activity, are readily recognised. One must be aware, however, that aEEG is still a simplified method for monitoring general cerebro-electrical background activity. For this reason it is advised that at least one standard EEG should be recorded when monitoring infants (7).

We used aEEG to monitor brain function following asphyxia (7). Several studies have shown that aEEG is an accurate measure to predict outcome even during the very first hours of extrauterine life. A normal voltage background pattern appearing within the first six h of life after birth asphyxia in term newborns predicts a normal outcome. Neurological outcome is impaired if a low voltage background pattern (BS, CLV or FT) persists (1, 8-11). Apart from the usefulness of the background pattern in predicting outcome, it is also a useful method for selecting those infants who might benefit from intervention after birth asphyxia (8). The usefulness of the aEEG in predicting neurological outcome in preterm infants is less clear, but preterm infants with a large intraventricular haemorrhage and a low voltage aEEG may have an impaired outcome as well (12). Considering the importance of the background activity for clinical purposes, it is important to be aware of certain pitfalls in interpreting aEEGs. In the present study we demonstrated that these pitfalls are drifting of the baseline of the aEEG due to HFOV, muscle artefacts and ECG. The higher baseline may lead to a more favourable interpretation of the aEEG and therefore one might assume the infant's neurological outcome to be more promising than in fact it is.

Amplitude-integrated EEG is also used to monitor sick newborn infants for seizures and to assess the effect of anti-epileptic treatment (13, 14). Earlier aEEG studies estimated that more than 50% of seizures are subclinical (13,15).. This may be important for assessing the risk for epilepsy later in life (15). It is still open to debate whether subclinical seizures should be treated. In our unit we treat subclinical seizures if they are repetitive with a frequency of at least three per hour, and in case of status epilepticus. When subclinical seizures are short and focal it can be difficult to recognise seizures on the aEEG trace (16). Reviewing suspicious aEEG traces with the simultaneously recorded single-lead EEG improves accurate detection of seizures. Epileptic discharges can either be confirmed or ruled out if seizure-like activity on the aEEG is the result of muscle artefacts. The simultaneously recorded single-lead EEG thus helps to prevent unnecessary administration of anti-epileptic drugs.

Conclusions

Although a simultaneously recorded single-lead EEG cannot replace a twenty-lead EEG, it can help to interpret the aEEG more accurately, both with respect to background patterns and epileptic activity.

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CHAPTER 9

Summary, general discussion, and future perspectives

Summary, general discussion, and future perspectives

Continuous brain monitoring has become part of routine neonatal care in a large number of neonatal intensive care units, all around the world. We described the two techniques that are used nowadays in neonatal intensive care for brain monitoring in the introduction of this thesis: amplitude-integrated EEG (aEEG) and near infrared spectroscopy (NIRS).

In **part 1** of this thesis, we present studies concerning the use of aEEG in term newborns. Since aEEG was introduced into the neonatal intensive care units, it has mainly been used in term newborns following perinatal asphyxia. Low voltage background patterns are related to poor neurological outcome or death (1-3). It is possible to predict neurological outcome on the basis of aEEG as early as six hours following perinatal asphyxia (4). In *chapter 2* we investigated the longitudinal course of aEEG in the first 72 hours following perinatal asphyxia. We showed that normalisation of the background pattern is not uncommon; it appeared in 40% of infants with a low voltage background pattern (BS, CLV, FT). This normalisation of the background pattern was related to a good outcome. This has now also been shown by others (5). Even the most severely abnormal background patterns, such as CLV and FT, can normalise within the first 24 hours. This occurred in 9% of these cases and was associated with a good outcome in 83% This data emphasizes the fact that it is important to continue aEEG monitoring beyond the first day of life.

Our data was obtained before hypothermia was introduced as neuroprotection following perinatal asphyxia (6, 7). Nowadays, infants that suffered perinatal asphyxia are treated with therapeutic hypothermia. Therapeutic hypothermia is the first therapy that proved to improve outcome in infants with encephalopathy due to perinatal asphyxia. It has been reported that the predictive value of aEEG is reduced in the case of therapeutic hypothermia (8). Normalisation of severely abnormal background patterns to CNV or DNV is delayed and sleep-wake cycles appear at a later stage. Nevertheless, aEEG remains an important tool to monitor brain function following perinatal asphyxia.

In *chapter 3,* we studied the aEEG in relation to outcome in infants with neonatal sepsis and meningitis. Infants with a sepsis or meningitis can be critically ill and sometimes need intensive care to support their vital functions. The most severely ill infants need artificial ventilation and/or circulatory support. These infants are at risk for neurological injury, which may be based on several mechanisms. This includes circulatory failure with decreased cerebral blood flow, poor cerebral oxygenation due to hypoventilation and respiratory failure, and brain damage due to inflammatory processes. Clinical assessment is often difficult because of the use of sedatives and neuromuscular blocking agents. In addition, sepsis in neonates is frequently accompanied by transient hypotonia, lethargy, and hypokinesia. The results of our study in *chapter 3* indicate that severely abnormal background patterns (FT, CLV and BS) are not uncommon in these infants, and that the risk for an adverse outcome is much

higher when such a background pattern persists for 24 hours. Sleep-wake cycling (SWC) appeared more frequently in infants with a good outcome. Clinical seizures were frequent and appeared in 53% of the infants. Electrographic seizure activity, however, was only present in 23% of the infants, and this was indicative of an adverse outcome. Half of the infants with clinical seizures without evidence of electrographic seizure activity had received anti-epileptic drugs prior to aEEG recording. The difference between clinical observation and aEEG can, therefore, be explained as successful treatment. Another explanation may be the fact that it is very difficult to diagnose seizures clinically (9). Our study underlines the necessity of aEEG monitoring as early as possible following the start of the disease and the need to continue monitoring for longer periods. In this way aEEG is an adjunct to clinical observation and can be helpful in predicting neurological outcome. To become, and remain a valuable tool in the neonatal intensive care, some of the hallmarks of using CFM are: constant education of the nursing staff and the medical staff, collaboration with clinical neurophysiologists and pediatric neurologists.

In *chapter 4,* we describe a cohort of infants diagnosed with a congenital heart disease (CHD). All infants had a ductus dependent CHD and were treated with prostaglandin E_1, in order to open the ductus arteriosus and to restore oxygenation and circulation. Infants who have a CHD are at risk of brain damage and consequently neurological impairment. Impaired cerebral oxygenation and perfusion most probably play a role in the evolution of this brain damage. In that case, abnormalities of cerebral electrical activity may indicate an impaired cerebral oxygenation and or perfusion. We divided our two groups: a group of infants with a duct dependent systemic circulation (acyanotic CHD), and a group of infants with a duct dependent pulmonary circulation (cyanotic CHD). The majority of infants in both groups of CHD had abnormal aEEG background patterns prior to surgery. Following reopening of the ductus arteriosus, mildly abnormal background patterns changed into normal background patterns within the first days. Electrographic seizure activity was more frequent in infants with an acyanotic CHD and correlated with more severe metabolic acidosis and multi-organ failure. These findings indicate that continuous monitoring by means of aEEG helps to identify electrographic seizure activity in case of CHD. For this reason, aEEG is very useful as a first screening tool to evaluate brain injury prior to surgery.

aEEG may also help to evaluate the timing of the injury. Preoperative brain injury in infants with CHD can already evolve *in utero*. Injury may also develop in the neonatal period following closure of the ductus arteriosus, intra-operatively and post-operatively. At all three stages, aEEG may help to evaluate brain function. Particularly in the postoperative stage, when neurological condition is often influenced by medication, aEEG may help to monitor background pattern and (silent) seizure activity. In this way, additional brain injury caused by ongoing seizure activity may be prevented. A new development is the combination of aEEG recording and monitoring of cerebral oxygenation, using NIRS. With this combined monitoring, more information on cerebral oxygenation and perfusion may be gathered.

Now that brain monitoring has become an integrated part of neonatal intensive care, aEEG and NIRS are also increasingly used in preterm infants.

In **part 2** we present studies concerning brain monitoring in preterm infants. There are some studies that indicate that aEEG may be helpful in predicting neurological outcome in high-risk preterms suffering intraventricular hemorrhages or aEEG patterns depend largely on postnatal and gestational age (16-20). Differentiation between background patterns at different gestational ages is difficult, because of the fact that preterm infants have profound discontinuous background patterns. Discontinuity decreases with increasing postnatal and gestational age. Therefore, it may be useful to have a more quantitative analysis. This might help comparing various aEEG patterns between different preterm infants. With the evolution of digital CFM equipment, quantification of the aEEG has become possible. We used a digital CFM device that comprises software facilitating computation of the aEEG amplitude centiles. We were interested whether other factors besides intraventricular hemorrhages, gestational age, and postnatal age do influence background activity in preterm infants. In *chapter 5* we studied a cohort of preterm infants that had daily aEEG recordings during the first 5 days after birth. aEEG was analysed using pattern recognition and calculating the centiles of the amplitude. For pattern recognition, we used the so-called Burdjalov-score (16), based on lower margin of the amplitude, bandwidth, and sleep-wake cycling. The assessment of aEEGs by calculating amplitude centiles proved to be a valid method, with a strong correlation with Burdjalov-scores. Postnatal age did not affect electro-cortical activity during the first five days after birth. As has been shown by others, electro-cerebral activity was clearly affected by gestational age. We also found that electro-cerebral activity of preterm infants was influenced negatively by the severity of illness of the preterm infant. This effect was the most apparent on the first day after birth. We found that particularly two aspects of severe illness, i.e. a low 5 minute Apgar score, and low blood pressure, had a suppressive effect on aEEG activity. This data indicates that it is important to monitor for a longer period of time, so that transient influences on electro-cerebral activity can be fully understood. The analysis by amplitude centiles also provides a more detailed analysing method for aEEG. In this way, numerous factors that can potentially influence brain activity can be analysed in a more robust manner than compared to analysis by pattern recognition.

In *chapter 6* we reported on the relationship between electro-cerebral activity and cerebral oxygenation in preterm infants of less than 32 weeks of gestation. Infants were prospectively monitored during the first two weeks of life. We analysed aEEG by calculation of aEEG amplitude centiles. With increasing gestational age, electro-cerebral activity matured, i.e. the 5th amplitude centile increased and the 95th centile decreased. There was a close relationship between electro-cerebral activity and oxygen consumption: the maturing of electro-cerebral activity was accompanied by an increased fractional tissue oxygen extraction (FTOE), indicating an increase in oxygen consumption. In the second week of life, the 5th aEEG amplitude centile

increased. This increase in electro-cerebral activity was accompanied by an increase of the oxygen consumption. However, oxygen consumption also increased with increasing postnatal age independently from more mature aEEG activity. In addition, oxygen consumption was influenced by hemoglobin level. We reported earlier that cerebral oxygenation in preterm infants may be at risk when Hb levels decrease below 6 mmol/l (21). Following a blood transfusion, cerebral oxygenation improved within one hour. Combining measurements of FTOE and electro-cerebral activity may be a useful predictor of brain function in high-risk infants. If so, it may help to treat preterm infants more accurately. A combination of high FTOE and low electro-cerebral activity may reflect impairment of cerebral oxygenation or perfusion and then serve as an indication for clinicians to focus on preserving oxygen supply to the brain in order to limit brain damage. It also helps to identify disturbances of brain perfusion during, or preceding, for example (cardio)surgery. A combination of low electro-cerebral activity, together with low FTOE, may indicate lower cerebral metabolism. This may be a result of neuronal loss following perinatal asphyxia (22). It may also be a result of adequate sedation in critically ill infants. Once again, it emphasizes the need for longitudinal recordings, for clinicians to fully understand the effect of therapies and changes in clinical conditions that take place. Further research is needed to clarify the possibilities, advantages, and pitfalls of this combined monitoring in both term and preterm infants.

In **part 3** of this thesis, attention is paid to factors that could affect the reliability of the aEEG. aEEG monitoring is used for several purposes. First of all, it is used to record electro-cerebral background activity. Together with physical examination and neuro-imaging it is used to assess neurological condition following perinatal asphyxia, in particular with emphasis on long term outcome. We have shown that aEEG monitoring is also useful in other groups of term infants in evaluating brain function (23-25).

One of the factors that can influence background pattern of aEEG is medication (26). In *chapter 7* we report about the influence of midazolam. Midazolam very effectively treated status epilepticus and repetitive seizures. Midazolam in high doses did lead to high serum levels, and this was accompanied by a burst suppression (BS) pattern on aEEG. These high serum levels are probably caused by a reduction of metabolism (liver) and clearance rates (kidneys) in critically ill newborns. Persistence of BS following perinatal asphyxia is associated with poor neurological outcome. Our data indicates that burst suppression on aEEG is of limited prognostic value when infants have high serumlevels of midazolam. To avoid high serum levels of midazolam, we recommend not increasing the dose of midazolam above 0.2 mg/kg/hr, and at the very least monitoring serum levels if higher doses are deemed necessary, or if there is burst suppression on the aEEG.

It is not known if the effects of midazolam are different while infants are undergoing therapeutic hypothermia. Metabolism and clearance rates might even be more

affected compared with infants that are normothermic. Perhaps it is not always necessary to start maintenance therapy, and a loading dose of midazolam is just as good in cessation of seizures. These questions remain to be answered.

Since aEEG monitoring has become a tool to monitor electrographic seizure activity (EA), it is very important that EA can be detected reliably. In *chapter 8* we reported about potential pitfalls in analysing both background pattern and EA and how more modern, digital CFM equipment can help to overcome these problems. The report clearly demonstrated that the simultaneously recorded original EEG helped to identify artifacts. Baseline drift could be attributed to either ECG, high frequency oscillation ventilation, or muscle activity. It was also helpful to distinguish between artifacts, muscle activity and seizures. Although a simultaneously recorded single-lead EEG cannot replace a twenty-lead EEG, it can help to interpret the aEEG more accurately, both with respect to background patterns and epileptic activity. Seizure detection remains a challenge, with or without a CFM (9, 27, 28). Hopefully, it becomes possible to integrate automated seizure detection software into digital CFMs, in order to decrease seizure burden (29;30).

Conclusion

In contemporary neonatology, some kind of cerebral monitoring should be part of standard care. A monitor has to be both easy to use and easy to interpret by all health workers working in the neonatal intensive care. Both aEEG and NIRS apply to this condition. As long as one is aware of the disadvantages of these monitors, both are an enrichment of neonatal intensive care. In order to overcome the shortcomings of aEEG monitoring, neonatologists, paediatric neurologists, and neurophysiologists must collaborate.

This thesis describes the usefulness of aEEG in a level III neonatal intensive care unit. We demonstrated that aEEG is also a valuable tool in monitoring brain function in term newborns with several clinical conditions that are potentially damaging to the brain. We also showed that continuing aEEG monitoring for longer time periods adds to the prognostic value of aEEG. It may create more knowledge about evolution and timing of brain injury. Combining both monitoring techniques may in future prove to be very useful in the treatment of several conditions in both term and preterm infants. It may improve clinical care. Older children may potentially benefit from continuous brain monitoring, particularly when requiring intensive care because of respiratory or circulatory insufficiency. Other examples in which aEEG and NIRS can improve clinical care are per operative and postoperative care of infants.

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Nederlandse samenvatting

In veel neonatale intensive care units is het continu monitoren van hersenactiviteit een standaard onderdeel van de bewaking van vitale functies van pasgeborenen geworden. In de introductie van dit proefschrift worden de beide technieken beschreven die gebruikt worden voor monitoren van hersenfunctie: amplitude-integrated EEG (aEEG) en near infrared spectroscopy (NIRS). Daarnaast wordt de opbouw van dit proefschrift uiteengezet. Het aEEG is een bewerkt 1-kanaals EEG en wordt gemeten met behulp van een cerebrale functie monitor (CFM). Na bewerking wordt het in de tijd gecomprimeerd en weergegeven op een semilogaritmische schaal. Een belangrijk onderdeel van de bewerking is het filteren van frequenties, frequenties onder 2 Hz en boven 15 Hz worden grotendeels uit gefilterd en hiermee wordt het optreden van artefacten geminimaliseerd. De semilogaritmische schaal zorgt ervoor dat hersenactiviteit van een lage amplitude relatief sterker wordt weergegeven. Van het aEEG kunnen verschillende aspecten worden beschreven, dit zijn: het achtergrondpatroon, slaap waak cycli en electrografische epileptische activiteit. Analyse van het achtergrondpatroon wordt met behulp van patroonherkenning gedaan. Verschillende patronen worden onderscheiden: continuous normal voltage (corticale activiteit met een voltage tussen de 10 en 25 (tot 50) μV; discontinuous normal voltage (discontinue activiteit met corticale activiteit voornamelijk boven de 5 μV); burst suppressie (BS, discontinue activiteit met perioden van erg lage hersenactiviteit (<5 μV) afgewisseld met 'bursts' van hoge activiteit); continuous low voltage (CLV, continu lage achtergrondactiviteit, rondom 5 μV); flat trace (FT, iso-elektrisch achtergrondpatroon, onder de 5 μV). Het achtergrondpatroon hangt sterk af van de zwangerschapsduur, het is meer uitgesproken discontinu bij een kortere zwangerschapsduur. Naast achtergrondactiviteit kunnen ook slaap-waak cycli (SWC) worden waargenomen, normaal gesproken ontstaan deze bij voldragen pasgeborenen binnen de eerste dag na geboorte. Als laatste kan het aEEG gebruikt worden voor het herkennen van electrografische epileptische activiteit.

De CFM werd in het verleden hoofdzakelijk gebruikt om hersenfunctie te registreren bij pasgeborenen die rond de geboorte een periode van asfyxie hadden doorgemaakt. Verstoring van zuurstof- en bloedvoorziening kan leiden tot verstoring van elektrische activiteit van de hersenen. In geval van lage achtergrondactiviteit (FT, CLV of BS), is de uitkomst op lange termijn slecht. Ook kunnen na asfyxie epileptische aanvallen ontstaan, deze worden niet altijd klinisch waargenomen. Met behulp van een CFM kunnen meer epileptische aanvallen worden opgespoord en kan het effect van behandeling geëvalueerd worden.

Een nieuwere methode van registreren van hersenfunctie is het meten van cerebrale zuurstofsaturatie met 'Near Infrared Spectroscopy' (NIRS). NIRS is een niet-invasieve methode en meet de zogenaamde regionale zuurstofsaturatie (r_cSO_2). R_cSO_2 geeft een gemiddelde zuurstofverzadiging weer in het vaatbed dat bestaat uit venen, arteriën en capillairen. De technologie maakt gebruik van het feit dat weefsels relatief transparant zijn voor licht in het bijna infrarode spectrum (600 to 900 nm). Een sensor meet de hoeveelheid gereflecteerde fotonen van 2 golflengtes (730 and 805 nm) en bepaald de absorptie van het onderliggende weefsel. Omdat geoxygeneerd

en gedeoxygeneerd hemoglobine verschillende absorptiespectra hebben, kan de zuurstofverzadiging van het onderliggende weefsel berekend worden. Door middel van de transcutane arteriële zuurstofverzadiging en r_cSO_2, kan de zogenaamde 'fractional tissue oxygen extraction' (FTOE) berekend worden. De FTOE geeft de balans tussen zuurstofaanbod en -verbruik weer.

In **deel 1** van dit proefschrift worden de studies over het gebruik van het aEEG bij voldragen pasgeborenen beschreven. Na de introductie van het aEEG in de neonatologie, is het in eerste instantie voornamelijk gebruikt bij voldragen pasgeborenen na een doorgemaakte perinatale asfyxie. Een laag gevolteerd achtergrondpatroon is gerelateerd aan een slechte neurologische uitkomst of overlijden. Al 6 uur na de geboorte, is het op basis van het aEEG mogelijk, het risico op een slechte neurologische uitkomst in te schatten.

In hoofdstuk 2 werd de betekenis van het longitudinale beloop van het aEEG gedurende de eerste 72 uur na asfyxie onderzocht. De studie toonde aan dat een afwijkend achtergrondpatroon nogal eens hersteld. In 40% van de kinderen met een laag achtergrond patroon (BS, CLV, FT) trad herstel op. Dit herstel van een afwijkend achtergrondpatroon ging gepaard met een normale uitkomst. Na ons is dit ook door andere groepen aangetoond. Deze studie onderstreept het belang van het voortzetten van de aEEG meting gedurende langere tijd.

In hoofdstuk 3 bestudeerden wij de relatie tussen het aEEG en neurologische uitkomst bij kinderen met een neonatale sepsis of meningitis. Pasgeborenen met een sepsis of meningitis kunnen ernstig ziek zijn en soms is dan intensive care noodzakelijk. De meest zieke kinderen worden opgenomen op een neonatale intensive care unit, omdat zij ondersteuning van vitale functies nodig hebben. Deze kinderen lopen het risico op het ontwikkelen van hersenschade, waarbij verschillende mechanismen een rol kunnen spelen. Klinische beoordeling is soms moeizaam, omdat kinderen worden behandeld met sedativa of spierrelaxantia. De studie toont aan dat ernstig afwijkende achtergrondpatronen (FT, CLV en BS) veelvuldig voorkomen en dat bij persisteren van een dergelijk afwijkend achtergrondpatroon gedurende langer dan 24 uur, het risico op een afwijkende utkomst vele malen hoger is. SWC waren frequenter aanwezig bij kinderen met een gunstige uitkomst. Klinische convulsies kwamen frequent voor, bij 53 % van de kinderen. Electrografische epileptische activiteit kwam slechts bij 23% van de kinderen voor. Het optreden van electrografische epileptische activiteit was gerelateerd aan een afwijkende uitkomst. De helft van de kinderen waarbij de klinische convulsies niet gepaard gingen met afwijkingen op het aEEG, waren al met anti-epileptica behandeld voordat gestart werd met registratie van het aEEG. Het verschil tussen klinische observatie en aEEG registratie kan dus het gevolg zijn van succesvolle behandeling. Een andere verklaring is het feit dat het herkennen van klinische convulsies bij pasgeborenen erg moeilijk is. Het onderstreept de noodzaak tot registratie van het aEEG zo vroeg mogelijk in het ziektebeloop, en het nut van langdurige registratie. Op deze manier is registratie van het aEEG een aanvulling op klinische observatie en kan het behulpzaam zijn bij het voorspellen van de neurologische uitkomst in deze groep kinderen.

In het laatste hoofdstuk van dit deel van het proefschrift wordt een groep kinderen beschreven met een aangeboren hartafwijking. Alle kinderen hadden een zogenaamde ductus afhankelijke hartafwijking en werden behandeld met prostaglandine, om zo de ductus van Botalli te openen en herstel van oxygenatie of circulatie te bewerkstelligen. Ook kinderen met een aangeboren hartafwijking lopen het risico op hersenschade en dientengevolge een ontwikkelingsachterstand. De groep kinderen werd verdeeld in twee groepen: een groep met een ductus afhankelijke lichaamscirculatie (acyanotische hartafwijking), en een groep met een ductus afhankelijke longcirculatie (cyanotische CHD). Bij kinderen met een acyanotische hartafwijking is er veelal sprake van een slechte lichaam- en/of hersendoorbloeding, terwijl er bij kinderen met een cyanotische hartafwijking sprake is van voornamelijk hypoxie. Onvoldoende oxygenatie en circulatie spelen zeer waarschijnlijk een rol in het ontstaan van hersenschade. Het tekort schieten van zuurstofvoorziening en doorbloeding van de hersenen kan gepaard gaan met een afwijkende hersenactiviteit. Het merendeel van de kinderen had voor de chirurgische ingreep die zij kregen al een afwijkend aEEG achtergrond patroon. Nadat de ductus van Botalli heropend was en er dus herstel was opgetreden van zuurstof- en bloedvoorziening, trad normalisatie van het achtergrondpatroon op gedurende de eerste dagen na opname. Electrografische epileptische activiteit kwam vaker voor bij kinderen met een acyanotische hartafwijking en was geassocieerd met een ernstigere metabole acidose en multi orgaan falen. Dit toont aan dat continue registratie van het aEEG helpt bij het opsporen van epileptische activiteit en dat het aEEG heel goed gebruikt kan worden om eventuele hersenschade bij kinderen met een hartafwijking te evalueren. Deze hersenschade kan op verschillende momenten ontstaan, al in de baarmoeder, maar ook in de fase vanaf geboorte tot aan het moment van operatie. Met aEEG kan het moment van ontstaan van hersenschade mogelijk beter geïdentificeerd worden. Ook zou het aEEG een eenvoudige monitor voor hersenactiviteit kunnen zijn tijdens de operatie en in de fase na operatie, wanneer de klinische observatie sterk beïnvloedt wordt door narcose en eventuele andere medicatie.

In het **tweede deel** van het proefschrift worden twee studies met betrekking tot te vroeg geboren kinderen gepresenteerd. Omdat aEEG registratie meer en meer onderdeel is geworden van de standaard zorg op een neonatale intensive care, worden deze technieken ook veelvuldig gebruikt bij prematuur geboren kinderen. De neurologische uitkomst van premature pasgeborenen voorspellen is veel moeilijker. Hiervoor wordt o.a. gebruik gemaakt van beeldvorming en observatie van spontane bewegingendpatroon met toename van zwangerschapsduur, verandert het ook met toename van postnatale leeftijd, het wordt meer continu. Een kwantitatieve analyse van het aEEG schept de mogelijkheid om het aEEG van verschillende groepen patiënten nauwkeuriger te vergelijken. Met de ontwikkeling van digitale CFMs is dit mogelijk. Wij gebruikten een digitale CFM die software bevatte waarmee de percentielen van de aEEG amplitude berekend worden. We wilden onderzoeken of behalve grote hersenbloedingen, zwangerschapsduur en postnatale leeftijd, ook andere factoren van invloed waren op de hersenactiviteit.

In hoofdstuk 5 onderzochten we een groep premature pasgeborenen, bij wie gedurende de eerste vijf levensdagen dagelijks een aEEG registratie was verricht. Het aEEG werd op 2 manieren geanalyseerd. Ten eerste met behulp van patroonherkenning en het opmaken van de zogenaamde Burdjalov-score. Deze score is gebaseerd op de hoogte van de ondergrens, de bandbreedte van de aEEG amplitude en het optreden van SWC. Daarnaast werd het aEEG geanalyseerd met behulp van de berekening van de percentielen van de aEEG amplitude. De analyse met behulp van percentielen bleek betrouwbaar te zijn, er was een sterke correlatie tussen de percentielen en de Burdjalov-scores. Er was geen invloed van postnatale leeftijd op het aEEG gedurende de eerste vijf levensdagen. Wel werd een duidelijk effect van zwangerschapsduur gevonden, zoals eerder door anderen was aangetoond. Daarnaast was er een duidelijk effect van ernst van ziekte op hersenactiviteit: hoe hoger de ziektescore, hoe lager de hersenactiviteit. Dit effect was het sterkste op de eerste dag na geboorte. Twee aspecten van ziek zijn, een slechtere start met een lagere Apgar score en lage bloeddruk, hadden een negatief effect op de hersenactiviteit. Opnieuw toont dit aan dat monitoren gedurende langere tijd (dagen) meer informatie geeft, zodat tijdelijke invloeden op hersenactiviteit beter begrepen kunnen worden. De analyse met behulp van de berekening van percentielen van de aEEG amplitude helpt hierbij en maakt het mogelijk het aEEG meer gedetailleerd te analyseren.

In hoofdstuk 6 wordt de relatie tussen elektrische hersenactiviteit en zuurstofverzadiging van de hersenen beschreven bij premature pasgeborenen met een zwangerschapsduur onder de 32 weken. Bij deze kinderen werden zowel aEEG als NIRS metingen verricht gedurende de eerste twee levensweken. Het aEEG werd geanalyseerd door middel van de in hoofdstuk 5 beschreven methode. Met toename van de zwangerschapsduur was er een "rijping" van de hersenactiviteit, de 5^{e} percentiel van de aEEG amplitude nam toe en de 95^{e} nam af. Gedurende de tweede levensweek was er een toename van de 5^{e} percentiel van de aEEG amplitude, deze toename ging gepaard met een toename in zuurstofverbruik. Er was sprake van een sterke relatie tussen de hersenactiviteit en zuurstof consumptie, de zuurstofconsumptie nam toe met rijping van de elektrische hersenactiviteit. Daarnaast was het zuurstofverbruik ook afhankelijk van het hemoglobinegehalte, hoe lager het hemoglobinegehalte hoe hoger het cerebrale zuurstofverbruik. Het combineren van de beide methodes kan eventueel nuttig zijn bij het voorspellen van prognose en bij een nauwkeurigere behandeling van premature pasgeborenen. Opnieuw wordt het nut van longitudinale metingen onderstreept, op verschillende momenten is er invloed van verschillende factoren.

In **deel 3** wordt stilgestaan bij factoren die de betrouwbaarheid van het aEEG kunnen beïnvloeden. aEEG registratie wordt gebruikt voor verschillende doeleinden: voorspellen van neurologische uitkomst, monitoren van epileptische activiteit. In hoofdstuk 7 wordt de invloed van midazolam beschreven. Midazolam was zeer effectief voor de behandeling van een status epilepticus. Er ontstonden echter zeer hoge bloedspiegels van midazolam, waarschijnlijk het gevolg van verminderd metabolisme in de lever en lagere klaring door de nieren, zoals gezien wordt bij kritiek zieke pasgebo-

renen. Deze hoge bloedspiegels van midazolam gingen gepaard met een BS patroon op het aEEG. Indien BS na asfyxie langdurig blijft bestaan is dit geassocieerd met een slechte neurologische prognose. De invloed van midazolam maakt het aEEG als voorspeller van prognose daarom minder betrouwbaar. Om hoge bloedspiegels te voorkomen wordt geadviseerd om midazolam niet verder op te hogen dan 0,2 mg/kg/u, en om op zijn minst bloedspiegels te controleren als het aEEG een BS patroon toont.

Daarnaast wordt het aEEG gebruikt voor het detecteren van epileptische activiteit. Het is daarom belangrijk dat deze detectie betrouwbaar is, zodat overbehandeling voorkomen wordt.

In hoofdstuk 8 wordt een drietal casus beschreven, waarbij valkuilen bij het beoordelen van het aEEG voor kwamen. Het betrof zowel analyse van het achtergrondpatroon, als analyse van epileptische activiteit. Met modernere, digitale apparatuur wordt het mogelijk deze problemen te voorkomen. Met het beoordelen van het gelijktijdig geregistreerde oorspronkelijk EEG signaal was het mogelijk artefacten te identificeren. Het ECG, hoog frequente beademing en spierartefacten kunnen allen leiden tot het omhoogdrijven van de ondergrens. Met het originele EEG signaal was het ook mogelijk om onderscheid te maken tussen epileptische activiteit, artefacten en spieractiviteit.

Samenvattend laat dit proefschrift zien dat registratie van het aEEG niet alleen waardevol is bij pasgeborenen met een doorgemaakte asfyxie, maar ook heel waardevol kan zijn bij andere groepen kritiek zieke pasgeborenen. Het langdurig registreren van het aEEG, vergroot de prognostische waarde en geeft ook inzicht in ontstaanswijze van hersenschade en de invloed van verschillende factoren. Het digitale tijdperk vergroot de mogelijkheden van het aEEG, beoordeling wordt betrouwbaarder, en ook het monitoren van premature pasgeborenen wordt waardevoller. De combinatie met monitoren van de zuurstofverzadiging van de hersenen vergroot het inzicht en schept nieuwe mogelijkheden qua diagnostiek en mogelijk ook qua behandeling. Het monitoren van hersenfunctie is niet meer weg te denken uit de moderne neonatologie.

Dankwoord

Een proefschrift gaat niet zonder dankwoord. Als nuchtere Fries moet je natuurlijk oppassen niet een te onderkoeld dankwoord te schrijven: *it koe minder.*

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Curriculum Vitae

Henk ter Horst werd op 21 april 1967 te Sneek geboren. Hij is de eerste van een tweeling, zijn tweelingbroer is Maarten ter Horst. In 1985 behaalde hij het eindexamen VWO aan de Rijks Scholen Gemeenschap te Sneek. In datzelfde jaar verhuisde hij naar Amsterdam, waar hij geneeskunde ging studeren aan de Universiteit van Amsterdam. Tijdens de studie geneeskunde was hij secretaris van de stichting Medische Onderwijsstages in Ontwikkelingslanden (MOSO)-Exchange. In 1989 volgde hij de Primary Health Care Field Rotation aan het Muhimbili Medical Centre, University of Dar Es Salaam te Tanzania. Tijdens deze Primary Health Care Field Rotation werd onderzoek verricht naar het gebruik van voorbehoedsmiddelen door middelbare school scholieren in Bagamoyo. In 1990 en 1993 behaalde hij aan de Universiteit van Amsterdam respectievelijk zijn doctoraal en artsexamen.

Na het behalen van zijn artsexamen werkte hij gedurende 2 jaar als arts-assistent in het Reinier de Graaf Ziekenhuis te Delft, waarna hij terugkeerde naar Amsterdam, om in het Academisch Medisch Centrum (AMC) als arts assistent te gaan werken op de afdeling neonatologie. In 1996 startte hij met de opleiding tot kinderarts in het Emma Kinderziekenhuis/AMC (opleider prof. dr. H.S.A. Heymans), het perifere deel van de opleiding werd gevolgd in het Medisch Centrum Alkmaar (opleider P.P.M. Schilte). Vanaf 1 april 2001 is hij geregistreerd als algemeen kinderarts.

In februari 2001 startte hij met de subspecialistische opleiding tot kinderarts-neonatoloog (opleiders prof. dr. S.B.A. Bambang Oetomo en prof. dr. A.F. Bos), welke eind 2003 werd afgerond. Tijdens de opleiding startte hij, naast de klinische werkzaamheden, met klinisch georiënteerd wetenschappelijk onderzoek, dit resulteerde in de totstandkoming van dit proefschrift. Sinds de afronding van de opleiding tot kinderarts-neonatoloog, is Henk staflid van de sectie Neonatologie van het Beatrix Kinderziekenhuis.

Henk woont samen met Yvonne Jeucken. Samen zijn zij de trotse ouders van een dochter (Eke) en een zoon (Jurre).

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CHAPTER 4

Amplitude integrated electroencephalographic activity in infants with congenital heart disease before surgery.

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Abstract

Background: Infants with congenital heart disease (CHD) are at risk for brain injury. An accurate tool to monitor brain function is amplitude integrated EEG (aEEG). It records both background patterns and electrographic seizure activity (EA).
Aims: Our aim was to determine aEEG patterns in infants with CHD and to determine the differences between infants with a cyanotic or an acyanotic CHD.
Study design and subjects: Sixty-two full term newborns had either a cyanotic CHD (transposition of the great arteries (n= 24)) or an acyanotic CHD (hypoplastic left heart syndrome (n= 26), critical aortic valve stenosis (n= 1) or aortic coarctation (n= 11)). The background patterns, sleep-wake cycling (SWC), and EA were assessed. The first 72 hours after starting prostaglandin E_1-therapy were used for analysis.
Results: The background patterns were mildly abnormal in 45% of the infants and severely abnormal at some point during the recording in 14% of the infants. We found no differences in background patterns between the two groups. EA was present in 12 (19%) infants. EA was more frequent in infants with acyanotic CHD (OR 9.4, 95% CI 1.1-78, p= 0.039). SWC was equally frequent in infants with cyanotic and infants with acyanotic CHD. A severely abnormal aEEG and EA were associated with more profound acidosis.
Conclusions: Before surgery the majority of infants with a CHD had an abnormal aEEG. aEEG helped to identify EA and it was a useful tool to evaluate brain function prior to surgery in CHD.

Introduction

Mortality in infants with congenital heart disease (CHD) has decreased over the last few decades due to improved diagnostic and therapeutic options. With this growing number of surviving infants neurological morbidity due to CHD, however, is increasing (1-3). Infants may have learning disabilities, seizures, cerebral palsy, hearing and speech problems, and motor problems (2, 4). Previous research focused on intra-operative factors, especially techniques such as cardiopulmonary bypass and deep hypothermia as a reason for brain injury (5). It is becoming increasingly clear, however, that the development of neurological morbidity in infants with CHD is a complex interaction between preoperative, intra-operative, and postoperative events (1, 6). Some data even suggest evolution of brain injury in utero (7, 8). In infants with CHD closure of the ductus arteriosus can result in hypoxemia or ischemia, and leads to decreased oxygen delivery to the tissues. Reduction in oxygen delivery initiates the shift to anaerobic metabolism which results in rapid depletion of energy reserves, accumulation of lactic acid, and the inability to maintain cellular functions. This energy failure will eventually cause neuronal cell death in the developing brain.

A method to determine the presence and severity of hypoxic ischemic encephalopathy is cerebral function monitoring (CFM). CFM is a bed-side tool that continuously monitors electro-cerebral activity through biparietal electrodes. It is used increasingly in neonatal intensive care units, particularly following perinatal asphyxia, in which case it has a high prognostic value for neurodevelopmental outcome (9-11). Low voltage aEEG patterns are associated with poor neurological outcome or death, even as early as 3 to 6 hours after birth (11). Previous studies showed that the background pattern of the aEEG correlates well with conventional EEG (12, 13). It is also possible to monitor epileptic activity using a CFM. A limitation is, however, that short and focal seizures may be missed (13). Non-expert users may miss a substantial part of electrographic seizure activity (14). Proper interpretation of electrographic seizures can be enhanced by simultaneously recording a single channel EEG (15, 16).

The question arises whether aEEG is helpful to determine brain hypoxia and ischemia in newborns with CHD. The effects on cerebral metabolism may differ between the different types of CHD. In newborn piglets, for instance, hypotension has a more profound effect on cortical electrical activity measured with a CFM than hypoxemia (17). Children with acyanotic CHD are more likely to develop neurological abnormalities than children with cyanotic defects (6, 18). For this purpose we also differentiated between two types of congenital heart disease. The first group of infants had a ductus dependent systemic circulation (acyanotic CHD) and the second group had a ductus dependent pulmonary circulation (cyanotic CHD). In infants with acyanotic CHD inadequate perfusion of the brain and other organs predominates. In infants with a cyanotic CHD hypoxemia occurs.

The aim of our study was to determine background patterns of aEEG, electrographic seizure activity, and sleep-wake cycling (SWC) in infants with a congenital heart defect prior to surgery. We also investigated whether there was a difference between

the aEEGs of infants with a cyanotic and an acyanotic CHD. We hypothesized that aEEG patterns in infants with acyanotic heart disease were more abnormal than those of infants with a cyanotic CHD.

Methods

Study population

This retrospective study was performed at the neonatal intensive care unit (NICU) of University Medical Center Groningen, the Netherlands. From our medical database we identified all infants that had been admitted with a CHD between March 2000 and March 2009 and that had been monitored with a cerebral function monitor (CFM). In our NICU aEEG recordings is routine procedure in all critically ill infants. The aEEG recordings were interpreted by the attending neonatologist.

Infants with a gestational age of less than 36 weeks were excluded. Since we were interested in differences between infants with a cyanotic and acyanotic heart disease we only selected those infants with transposition of the great arteries (TGA), hypoplastic left heart syndrome (HLHS), aortic valve stenosis (AS), and aortic coarctation (CoA). The final cohort consisted of 62 infants. Twenty-four infants had a TGA. Of the 38 infants with a ductus dependent systemic circulation 26 had HLHS, one had a critical AS, and the remaining 11 infants had CoA.

We obtained the clinical information on the infants from their medical records. This included Apgar scores, gestational age, birth weight, circulatory and respiratory parameters, blood gas analyses at the time of admittance, the presence of multi-organ failure, neurological examination, the presence of clinical seizures, use of sedative drugs, and data on neuroimaging. Following the treatment with prostaglandin E_1 infusion reopening of the ductus arteriosus was verified by echocardiography.

The study was approved by the review board of University Medical Center Groningen.

The aEEG recordings

The mean duration of the aEEG recordings was 54 h (6-155 h). The time that infusion of prostaglandin E_1 was first started was considered t_0. We took the moment prostaglandin E_1 was started, because just previously brain oxygenation and/or perfusion may have become impaired. This is comparable to the timing of perinatal asphyxia, where the hypoxic ischemic event takes place around birth.

The aEEG recordings commenced at a mean of 7.5 h (range 0-41) following prostaglandin E_1 infusion. Eighty percent of the recordings commenced within 12 h following prostaglandin infusion and 94% within 24 h. We analyzed the first 72 hours of the aEEG recordings following t_0. Biparietal needle electrodes (P3 and P4 according to the international 10-20 system of electrode placement) were used for the recordings and for the duration of each recording the impedance was less than 5 kΩ.

Assessment of the aEEG recordings

The aEEGs were recorded by either one of two cerebral function monitors: the analog Lectromed® Multitrace 2 or the digital Olympic® CFM 6000. When the Lectromed® Multitrace 2 monitor was used, it was calibrated every 24 h. The nursing staff recorded all nursing and medical procedures, clinical seizures, and the administration of all medication.

The aEEG recordings were assessed by pattern recognition. The background patterns, EA, and the presence SWC were assessed. SWC was identified by an altered width of the amplitude of the tracing indicating different, alternating sleep stages. When the aEEG was recorded on the Olympic® CFM 6000 monitor, epileptic activity was confirmed by analysis of the simultaneously recorded single-lead EEG. We classified the different background patterns as continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage (CLV) or flat trace (FT), according to the criteria of Toet et al. (11). CNV was considered a normal background pattern, DNV as mildly abnormal while we considered the low voltage traces (BS, CLV, and FT) as severely abnormal.

Electrographic seizure activity (EA) was classified as:
Single seizure (SS). Single event of sudden sustained high cortical activity.
Repetitive seizures (RS). Repetitive events of sudden sustained high cortical activity.
Status epilepticus (SE). Repeated EA, resulting in a regular pattern of increased cortical activity (saw tooth pattern) lasting for more than 50% of a period of the aEEG.

All traces were analyzed by one expert investigator (HtH). He was blind to the type of CHD the infant had as well as any other information concerning the infant.

Statistics

SPSS software for Windows, version 16.0 (SPSS Inc. Chicago, Illinois) was used for all analyses. Spearman's rank order correlation coefficient was used to calculate the correlation between the aEEG data and the clinical condition of the infants. We used Fisher's Exact test to test the proportions of categorical data. Mean values were analyzed by an independent sample t-test in the case of a normal distribution, or with the Mann Whitney U test in the case of a non-normal distribution. We calculated odds ratios (ORs) by univariate and multivariate logistic regression to determine which factors were independently related to aEEG background patterns and the presence of EA. Only factors identified by the univariate analysis (with $p < 0.10$) were included in the multivariate model. A p-value of less than 0.05 was considered significant.

Results

Subjects

The clinical data of the study population are summarized in Table 1. The mean gestational age was 39.8 weeks (range 36-42.9). The mean birth weight was 3414

grams (range 2180-4700). Twenty three infants were admitted on the day of birth and the rest between one and 22 days after birth. None had perinatal asphyxia. In nine infants CHD had been diagnosed prenatally. All infants were treated with prostaglandin E_1 (0.025 to 0.1 µg/kg/min). Of the 24 infants with TGA, 17 (71%) received additional treatment with balloon atrial septostomy. The infants with acyanotic CHD had clinical conditions like a more profound acidosis and multi-organ failure. Within the group of infants with acyanotic CHD there was no difference in such clinical conditions between infants with HLHS and infants with CoA. The infants with CoA were admitted at a later postnatal age (7.9 versus 2.8 days, p= 0.001).

Table 1: patient characteristics

	TGA (N= 24)	HLHS/CoA/AS (N= 38)	*p*-value
GA, wks, mean (range)	39.5 (36-41.7)	39.9 (36-42.9)	NS
BW grams, mean (range)	3474 (2355-4575)	3361 (2180-4700)	NS
Apgar score at 1 min, mean (range)	7.4 (2-9)	8.5 (6-10)	0.004
Apgar score at 5 min, mean (range)	8.8 (7-10)	9.4 (6-10)	0.011
Day of admission, mean (range)	3.3 (1-22)	4.4 (1-21)	0.006
Need for MV, N (%)	21 (88)	34 (90)	NS
Need for inotropes, N (%)	13 (54)	10 (26)	0.034
Clinical seizures, N (%)	3 (13)	12 (32)	0.08
pH	7.26 (6.92-7.50)	7.1 (6.68-7.37)	0.001
BE, mmol/l, mean (range)	-5.9 (-21 - 1)	-16 (-29 - -2)	< 0.001
Lactate, mmol/l, mean (range)	5.0 (1.2-28.4)	10.3 (1.2-28.6)	0.008
Serum creatinine level, µmol/l (range)	72 (35-132)	126 (22-410)	0.001
ASAT, U/l,(range)	95 (32-264)	492 (35-3291)	0.001

TGA: transposition of the great arteries; HLHS; hypoplastic left heart syndrome; CoA: aortic coarctation; AS: aortic valve stenosis; GA: gestational age; BW; birth weight; MV: mechanical ventilation; BE: base excess; ASAT: aspartate aminotransferase; NS: not significant; N: number

The aEEG background pattern

Twenty-six infants (40%) had CNV during the entire recording. 45% of the infants had a mildly abnormal background pattern (DNV) at some point during the recording. The percentage of infants with CNV increased over time from 43% at t_6 to 63%, 84%, and 87% at t_{24}, t_{48}, and t_{72}. Severely abnormal background patterns (BS, CLV or FT) were present in nine of the 62 infants. Severely abnormal background patterns were equally frequent in infants with acyanotic CHD (16%) and infants with cyanotic CHD (13%). Within the group of infants with an acyanotic CHD, none of the infants with CoA had a severely abnormal background pattern. This difference was not significant. We found no relationship between the day of admission and the presence of

a severely abnormal background pattern at any time of the aEEG recording. In the 17 infants treated with balloon atrial septostomy, we found no change in background patterns within the first hours following the procedure.

Eight infants already had aEEG recordings started before the start of Prostaglandin E_1 infusion. None of these infants had a severely abnormal aEEG during the first 24 h. Five infants had CNV during their entire recording. The remaining three infants had DNV, which developed into CNV within 24 hours in two of the infants. In only one infant the background pattern worsened to BS following treatment of seizures. Even if CHD had been diagnosed prenatally, the background patterns were not different.

In some infants the aEEG recording was discontinued before 72 hours following prostaglandin E_1 infusion. Two infants died within 24 hours after admission. Of the remaining 60 infants aEEGs were recorded for at least 24 hours in 54 (88%) infants and for at least 48 hours in 36 (60%) infants. If an aEEG had been discontinued before 72 hours the background pattern showed CNV without evidence of EA.

Electrographic seizure activity

EA was present in 12 infants, one of whom had status epilepticus. One infant had a single seizure and the remaining ten infants had repetitive seizures. EA was more frequent in infants with acyanotic CHD with an OR of 9.37 (95% CI 1.12-78.2, p= 0.039). Only one infant with a cyanotic CHD had EA. This particular infant had an ischemic infarction on magnetic resonance imaging following balloon atrial septostomy. We found no difference in EA between infants with CoA (25%) and infants with HLHS (27%). The single infant with AS also had EA. We found no relationship between the day of admission and the occurrence of EA. None of the infants with a prenatal diagnosis of CHD had EA. This difference was not significant.

We found a strong correlation between EA and the presence of clinical seizures (r= 0.67, $p < 0.0001$). Ten of the 12 infants with EA had clinical seizures. The two infants with subclinical seizure activity did not receive anti-epileptic treatment. One had a single seizure and one showed repetitive seizure activity. An additional five infants were suspected of having clinical seizures without showing EA on aEEG. A full EEG was performed in four of these five infants. Three of them had focal electrographic seizure activity at the time of EEG recording, and one had no electrographic seizure activity. Four of them were treated with anti-epileptic drugs in accordance with our protocol. The drug of first choice was phenobarbitone with a maximum loading dose of 30 mg/kg. Three of the 15 infants that had received phenobarbitone also needed the second drug of choice to control their seizures (midazolam: loading dose 0.05 mg/kg, maintenance dose 0.15-0.2 mg/kg/h). None of the infants needed a third anti-epileptic drug.

Sleep-Wake cycling

SWC was present in 36 (58%) infants within 72 h following the start of prostaglandin E_1 infusion. There was no difference in postnatal age at admission between infants

with and without SWC (3.5 versus 4.6 days respectively). We found also no difference in the presence of SWC between infants with an acyanotic and a cyanotic CHD, nor in the time of onset (32.4 versus 30.3 h respectively). In the group of infants with an acyanotic CHD, SWC was more frequent in infants with CoA than in infants with HLHS (92% versus 48%, p= 0.013). If SWC was present in this group of infants, there was a trend towards earlier onset of SWC in infants with CoA in comparison to infants with HLHS (26 versus 38 h, p= 0.1).

Neurological examination
Sixteen infants had a normal neurological exam during admission, 18 infants were hypertonic and irritable, and 17 infants were hypotonic and lethargic. Five infants received neuromuscular blocking agents, and in another six infants neurological examination was unreliable because of high doses of morphine.

An abnormal neurological examination was more frequent in infants with an acyanotic CHD than in infants with a cyanotic CHD (53% versus 21%, p= 0.027)

Of the infants with a normal neurological examination eight had CNV and eight had DNV. One of these 16 infants had EA. Severely abnormal background patterns were more frequent in infants that were hypotonic and lethargic than in infants that were hypertonic and irritable (35% versus 0%, p= 0.008). EA on aEEG was also more frequent if infants were hypotonic and lethargic than in infants that were hypertonic and irritable (47% versus 11%, p= 0.027).

Of the 11 infants with an unreliable neurological examination because of morphine or neuromuscular blocking agents, three had a severely abnormal background pattern, and one had EA.

Neuroimaging
Cranial ultrasound was performed in 56 (90%) of the infants. Ultrasound was normal in 47 infants and showed signs of ischemia in 9 (16%) infants. There was a trend for more severely abnormal background patterns in case of an abnormal ultrasound (OR 5.4, 95% CI 0.96-30.1, p= 0.056). The same was true when the aEEG showed EA (OR 3.9, 95% CI 0.85-17.8, p= 0.079).

A MRI scan was performed in 17 (27%) of the infants. The majority of the infants (n= 15) with a MRI had an acyanotic CHD. Ten (59%) of these 17 infants had MRI scans with ischemic lesions or cerebral infarction. EA was present in 8 of the infants. There was an increased risk for an abnormal MRI if EA was present (OR 14, 95% CI 1.1-172, p= 0.039).

The relationship between the clinical conditions and the aEEG characteristics
Perinatal asphyxia was not present in any of the infants, none of the infants had an Apgar score below six at five minutes. Twenty-six infants received sedative drugs (morphine n= 20; morphine and midazolam n= 6). We did not find any relationship

Table 2: Relationship between clinical conditions and aEEG background pattern

	SA (9)	MA/No (53)	OR (95%CI)	*p*
GA, wks, mean (range)	49.2 (36-41)	39.8 (36-42.9)		NS
BW grams, mean (range)	3335 (2180-3920)	3428 (2355-4700)		NS
Apgar score at 1 min, mean (range)	7.7 (5-9)	8.2 (2-10)		NS
Apgar score at 5 min, mean (range)	8.6 (6-10)	9.3 (7-10)	0.50 (0.25-0.98)	0.046
Day of admission, mean (range)	2.9 (1-11)	4.1 (1-22)		NS
Acyanotic CHD, N (%)	6 (67)	33 (61)		NS
Need for MV, N (%)	9(100)	46 (87)		NS
Need for inotropes, N (%)	5 (56)	18 (34)		NS
Clinical seizures,n (%)	5 (56)	10 (19)	4.89 (1.12-21.3)	0.035
pH	7.02 (6.8-7.35)	7.18 (6.68-7.50)	0.014 (0.000-0.64)	0.029
BE, mmol/l, mean (range)	-19 (-29 - -4)	-11 (-28 - 1)	0.90 (0.83-0.98)	0.022
Lactate, mmol/l, mean (range)	16.1 (4.5-28.4)	7.5 (1.2-28.6)	1.12 (1.02-1.24)	0.024
Serum creatinine level, µmol/l (range)	122 (61-295)	98 (22-410)		NS
ASAT, U/l,(range)	240 (35-633)	342 (32-3291)		NS

SA: severely abnormal; MA: mildly abnormal; No; normal GA: gestational age; BW; birth weight; MV: mechanical ventilation; BE: base excess; ASAT: aspartate aminotransferase; NS: not significant; N: number.

between the type of background pattern and the treatment with sedatives. The presence and time of onset of sleep cycling were also not related to the treatment with sedative drugs.

The relationship between clinical conditions and aEEG background patterns is shown in Table 2. The background patterns of infants with more profound lactic acidosis were more severely depressed; pH and base excess were lower and lactate higher when a severely abnormal background pattern was present at any time during the recording period. Additional factors related to severely depressed aEEG background patterns were presence of clinical seizures and Apgar scores at five minutes. In the multivariate model we entered pH, presence of clinical seizures and Apgar scores at 5 minutes. Presence of clinical seizures (OR: 12.02 [95% confidence interval (CI): 1.51-96]; p= 0.019), pH (OR: 0.003 [95% CI: 0.000-0.35]; p= 0.017), and Apgar score at five minutes (OR: 0.27 [95% CI: 0.10-0.71]; p= 0.008) remained in the model, explaining 43.7% of the variance of normal and severely depressed aEEG background patterns.

The relationship between clinical conditions and EA is shown in Table 3. In the presence of EA on the aEEG recording both pH and base excess were lower and

aspartate aminotransferase and serum creatinine were higher if EA was present. Additional factors related to EA were presence of clinical seizures, the type of CHD, and Apgar scores at five minutes. In the multivariate model we entered pH, type of CHD, and Apgar scores at 5 minutes. Only Apgar scores at 5 minutes (OR: 2.69 [95% CI: 0.88-8.23]; p= 0.083) and pH (OR: 0.016 [95% CI: 0.000-0.62]; p= 0.027) remained in the model, explaining 25.0% of the variance of EA.

Table 3: Relationship between clinical conditions and electrographic seizure activity

	EA + (12)	EA – (50)	OR (95% CI)	p
GA, wks, mean (range)	40.2 (37-41.9)	39.6 (36-42.9)		NS
BW grams, mean (range)	3630 (2400-4700)	3367 (2180-4575)		NS
Apgar score at 1 min, mean (range)	8.4 (7-9)	8.0 (2-10)		NS
Apgar score at 5 min, mean (range)	9.7 (9-10)	9.1 (6-10)	2.98 (0.97-9.12)	0.056
Day of admission, mean (range)	4.4 (1-11)	3.8 (1-22)		NS
Acyanotic CHD, N (%)	11 (92)	28 (55)	9.37 (1.12-78.2)	0.039
Need for MV, N (%)	12 (100)	44 (86)		NS
Need for inotropes, N (%)	3 (25)	20 (39)		NS
Clinical seizures,n (%)	10 (83)	6 (12)	45.0 (6.6-266)	< 0.001
pH	7.03 (6.8-7.25)	7.18 (6.68-7.50)	0.012 (0.001-0.41)	0.014
BE, mmol/l, mean (range)	-20 (-29 - -12)	-10 (-28.2 - 1)	0.87 (0.80-0.95)	0.002
Lactate, mmol/l, mean (range)	12.8 (1.5-28.6)	7.7 (1.2-28.4)		NS
Serum creatinine level, µmol/l (range)	137 (61-295)	94 (22-410)	1.01 (1.00-1.02)	0.047
ASAT, U/l,(range)	1122 (50-3291)	186 (32-1005)	1.003 (1-1.006)	0.037

EA: electrographic seizure activity; OR: odds ratio; CI: confidence interval; GA: gestational age; BW; birth weight; CHD: congenital heart disease; MV: mechanical ventilation; BE: base excess; ASAT: aspartate aminotransferase; NS: not significant; N: number.

Discussion

Our study demonstrated that abnormal aEEG background patterns were frequent in infants with CHD. Almost 60% of the infants had a mildly abnormal (DNV) or a severely abnormal aEEG background pattern (BS, CLV, FT) at some point during the recording period. Fourteen percent had a severely abnormal background pattern at some point during the recording period. EA appeared in 19% of the infants.

To our knowledge this was one of the first studies to evaluate the use of aEEG in a large cohort of infants with CHD. Our study cohort is quite large in comparison to

previous studies on electro-cerebral activity in infants with CHD in the pre-operative phase. Previous studies reported on EEG. Their findings are largely comparable to ours (19, 20). In contrast to EEG, however, aEEG enables us to monitor and detect changes in electro-cortical activity over a longer period of time.

The lower electro-cortical activity can be explained by decreased cerebral blood flow (21). If auto-regulation of cerebral blood flow is impaired this effect may be even stronger. In our study, we noticed an improvement of background pattern, from DNV to CNV, following prostaglandin E_1 infusion. With prostaglandin E_1 infusion the ductus arteriosus reopens and oxygen delivery to the brain recovers, either by increased perfusion or increased oxygenation. As a result the electro-cortical activity can recover. The same occurs following reperfusion in perinatal asphyxia in which case even recovery of severely abnormal background patterns occurs after the reperfusion period (9, 22). Our study showed that aEEG is an adjunct diagnostic tool in critically ill neonates with CHD. It can be continued for longer periods, even during and following surgery. Since there is increasing evidence that the evolution of brain injury in infants with CHD already starts before birth, aEEG could help to understand the timing of the brain injury, especially if CHD is diagnosed prenatally.

The prevalence of pre-operative EA in our cohort was 19%. This percentage is comparable to the limited data available (19, 23). EA was more frequent in the infants with acyanotic CHD. This indicates that lower cerebral perfusion is more injurious to the brain than hypoxemia. This is consistent with animal experiments, where hypotension has a more profound effect on electro-cortical activity than hypoxemia (17). This is also consistent with observations in infants with acyanotic CHD, who are more likely to have an altered neurological status prior to surgery compared to infants with cyanotic CHD (6). Moreover, an increased incidence of ultrasound abnormalities has been reported in infants with acyanotic CHD (24, 25). EA may be a marker of neurological damage in infants with CHD (19). At the same time, EA may also cause additional brain damage (26). EA occurred frequently in infants with CoA. Since cerebral perfusion remained intact in infants with CoA we were puzzled by the high incidence of EA in infants with CoA. We speculate here that following treatment with prostaglandin E_1 in infants with CoA, the existing upper body hypertension dissolves and as a result there may be a period of relative under perfusion of the brain.

In contrast to the higher incidence of EA in infants with acyanotic CHD, we did not find significant differences in background patterns between infants with cyanotic and acyanotic CHD. This is in line with a report from Limperopoulos et al., who used conventional EEG (19).

Infants with severely abnormal aEEG background patterns and presence of EA had a more profound acidosis. In infants with duct dependent CHD, closure of the ductus arteriosus leads to impaired oxygen delivery to the brain and other organs. This may explain why infants with more severe acidosis, resulting from impaired perfusion and oxygenation, have more abnormal aEEG background patterns and EA. The

persistence of lactic acidosis following perinatal asphyxia is also correlated with more severely abnormal EEG and increased seizure burden (27).

SWC emerged equally often in infants with acyanotic and infants with cyanotic CHD. In the group of infants with acyanotic CHD, SWC appeared more frequently and earlier in the infants with CoA. Following perinatal asphyxia, early onset of SWC is an indicator of good prognosis (28). The earlier onset of SWC in the infants with CoA may indicate preserved brain function. In healthy full term infants SWC normally emerges within the first 12 hours after birth (29). The difference in emergence of SWC may, however, also be explained by the higher postnatal age at admission of the infants with CoA.

aEEG is an accurate tool for predicting neurological outcome in infants following perinatal asphyxia (9, 11). Because long term follow up was not part of this retrospective study, it was not possible to evaluate the predictive value of aEEG for neurological outcome in this group of infants. Nevertheless we believe that persistence of severely abnormal aEEG patterns before surgery is indicative of brain injury. A normal aEEG prior to surgery will however not guarantee a normal neurological development, because the complexity of surgery and complications during and following surgery can add to the development of brain injury. Therefore aEEG can be helpful in understanding the timing of brain injury in infants with CHD.

There were some limitations to our study. Due to a variety of reasons the aEEG recordings were not started at t_0 in all cases. Firstly, our unit is a referral hospital for congenital cardiac surgery, thus infants are transferred from a large region. Prior to a transfer to our hospital, infants are stabilized and prostaglandin E_1 infusion is started if CHD is suspected. Nevertheless, the majority of recordings did commence before 12 hours following treatment with prostaglandin E_1. Secondly, CFM equipment is limited and in some cases it was not available when an infant was admitted. Not all recordings were of the same duration. Some infants died within 72 hours of admission because they proved impossible to stabilize. The remaining infants with recordings that were stopped before 72 hours had CNV background patterns without evidence of EA. A final limitation of our study is that we do not have early postoperative and long term follow up. We were interested in aEEG prior to surgery as a marker of brain damage before surgery. Final neurological outcome is not only determined by preoperative brain damage, but also strongly dependent on complications during surgery and postoperative complications. These complications are largely dependent on the complexity of the surgery. In our opinion the aEEG can also be useful as a monitor following surgery, but we do not have these data. Postoperative aEEG and neurological follow up should be a part of future studies.

Conclusions

The majority of infants with CHD had abnormal aEEG background patterns. Mildly abnormal background patterns changed to normal background patterns within the

first days after reopening of the ductus arteriosus. Electrographic seizure activity was more frequent in infants with an acyanotic CHD. Depression of the background pattern and EA correlated with more severe metabolic acidosis and multi-organ failure. SWC was more frequent in infants with CoA in comparison to infants with a HLHS. Continuous monitoring by means of aEEG could help identify EA in case of CHD and it could be a first screening tool to evaluate brain injury prior to surgery.

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PART 2

Amplitude integrated EEG recordings in preterm newborns

CHAPTER 5

Amplitude-integrated electroencephalographic activity is suppressed in preterm infants with high scores on illness severity

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Abstract

Background: The neonatal acute physiology score, SNAP-II, reflects the severity of illness in newborns. In term newborns, amplitude integrated EEG (aEEG), is depressed following asphyxia. In preterm infants aEEG is discontinuous, and therefore more difficult to assess compared to term infants.

Aims: Our first aim was to investigate whether assessing aEEG amplitudes by calculating amplitude centiles was consistent with assessment by pattern recognition. Our second aim was to investigate whether the aEEGs of preterm infants were influenced by SNAP-II.

Study design and subjects: We recorded aEEGs in 38 infants with a mean gestational age of 29.7 weeks (26.0-31.8 weeks) during the first five days of life. The mean recording time was 130 minutes. The aEEGs were assessed by pattern recognition, by calculating Burdjalov score, and by calculating the mean values of the 5th, 50th, and 95th centiles of the aEEG amplitudes. Illness severity was determined within the first 24 hours.

Results: We assessed 151 recordings and found strong correlations between the 5th and 50th amplitude centiles and the Burdjalov scores (r= 0.71, p <0.001 and r= 0.47, p <0.001, respectively). The 5th and 50th amplitude centiles correlated with SNAP-II (r= -0.34, p <0.0001 and r= -0.27, p= 0.001). These correlations were the strongest on the first day of life (r= -0.55, p= 0.005 and r= -0.47, p = 0.018, respectively). The 5th and the 50th amplitude centiles were best predicted by gestational age, SNAP-II, and low blood pressure.

Conclusions: Severe illness as measured by the SNAP-II, and low blood pressure had a negative influence on the aEEGs of preterm infants.

Introduction

Neonatal intensive care involves continuous monitoring of vital signs such as heart rate, arterial oxygen saturation, and blood pressure. Means of assessing the neurological condition of an infant admitted to a neonatal intensive care unit (NICU) are limited. The standard diagnostic tools include clinical observations of general movements and cerebral ultra-sonography (1, 2). Another method of assessing brain function is to measure by means of an electro-encephalogram (EEG). It provides information about patterns of cerebral activity, such as the different stages of sleep and wakefulness, and reveals pathological processes like seizures (3).

In our NICU we use a cerebral function monitor (CFM) to continuously monitor electro-cerebral activity. The CFM displays a single channel EEG recorded through bi-parietal electrodes. It provides a simplified, time-compressed EEG, a so-called amplitude-integrated EEG (aEEG). aEEGs are interpreted by pattern recognition, which is, to some extent, a subjective method (4). A previous EEG study of preterm infants demonstrated that electro-cerebral activity varies with gestational age (5). With increasing gestational age the background activity changes from discontinuous normal voltage to continuous normal voltage and eventually to continuous normal voltage with sleep-wake cycling. Young preterm infants have profoundly discontinuous background patterns on both EEG and aEEG, often appearing as burst suppression (6-8). In preterm infants aEEGs are influenced by both gestational age and postnatal age (9). Based on the features of pattern recognition (continuity, cycling, lower border, and bandwidth of the aEEG amplitude) the so-called Burdjalov score can be calculated (8). This score increases with increasing gestational age. By using a digital device that computes the centiles of the aEEG amplitudes, the aEEGs can be quantified in more detail.

In full term infants aEEG traces are very effective in predicting neurological outcome at an early stage following perinatal asphyxia (10-13). Low voltage traces predict an impaired neurological outcome. Some studies indicated that aEEGs may be helpful in predicting neurological outcome in high-risk preterms with intraventricular haemorrhage (IVH) or post-haemorrhagic hydrocephalus (14-16). It is unknown whether the severity of illness in preterm infants influences their aEEGs. Illness severity can be assessed by the score for neonatal acute physiology, the SNAP-II score (17).

Our first aim was to investigate whether assessing the aEEG amplitudes by calculating amplitude centiles was consistent with calculation of the Burdjalov score. Our second aim was to investigate whether the aEEGs of preterm infants were influenced by the SNAP-II scores during the first five days of life. We hypothesized that the aEEGs were more discontinuous in case of high SNAP-II scores. In addition, we investigated which individual items of the SNAP-II score and the 5 minute Apgar score influenced the aEEGs.

Methods

This prospective observational study was performed at the NICU of the University Medical Center Groningen in the Netherlands from February 2006 to February 2007. All preterm infants with a gestational age of 26-32 weeks admitted on the first day of life were eligible for inclusion. Exclusion criteria were IVH exceeding grade II according to Volpe (18), chromosomal abnormalities, and severe congenital malformations. Infants with IVH exceeding grade II were excluded because electro cerebral activity may be negatively influenced in case of a large IVH (14). Because we had only one monitor available it was possible to make recordings in maximally 3 infants daily. Therefore it was not possible to include all preterm infants born and admitted during the study period. Selection of the included infants was, however, at random. Both parents gave their informed consent and the study was approved by the review board of Groningen University Medical Center.

Routinely, cranial ultrasound scans were made of all infants within 48 hours after birth.

Illness severity model

Neonatal illness severity was assessed by using the score for neonatal acute physiology II, SNAP-II (17). SNAP-II consists of six items: lowest mean blood pressure, lowest temperature, pO_2/FiO_2-ratio, lowest serum pH, occurrence of seizures, and urine output. The scores were obtained during the first 24 hours after birth.

Measuring the aEEGs

The aEEGs were measured daily during the first five days of life for approximately two hours. We used a digital CFM that was not commercially available at the time of the study. It consisted of an amplifier connected to a laptop computer that contained software for digital aEEG processing. In addition to the aEEG pattern, the original EEG was also displayed. The device recorded the aEEG through two electrodes: P3 and P4 position (international 10-20 system). The common electrode was placed anywhere on the body in a convenient position. We used neonatal ECG electrodes with a diameter of 15 mm (Neotrode II, Conmed, Utica, NY, USA). We used a digital DC common average reference amplifier (Porti-X by TMSi, Enschede, the Netherlands) comprising a high input impedance (> 2 GΩ) and a 22 bits sigma-delta Analog to Digital Converter with a resolution of 0.0715 μV per bit and a sample frequency of 500 Hz. The EEG electrodes were connected to the amplifier by means of shielded cables to prevent electrical noise and mains interference pick-up. Loss of electrode contact was sensed by the amplifier's input circuitry and signalled to the data acquisition software. Low (< 0.5 Hz) and high frequencies (> 25 Hz) were attenuated by first order high and low pass filtering. The EEG was subsampled at 200 Hz and stored on a hard disc and the aEEGs were processed at this subsample frequency.

The aEEG processor was constructed in software, and comprised a signal shaping filter, a semi-logarithmic rectifier, a peak detector and a smoothing filter. Its characteristics were similar to the CFM constructed and described by Maynard in

hardware (19). To gain more information the mean of the aEEG amplitude, and the mean peak and mean trough values, were computed and displayed. All values were filtered by box-car averagers with a time window of 60 seconds. The mean trough and mean peak values represented the 5th and 95th centiles of the aEEG amplitudes. Examples of aEEG traces with centiles are shown in Figure 1.

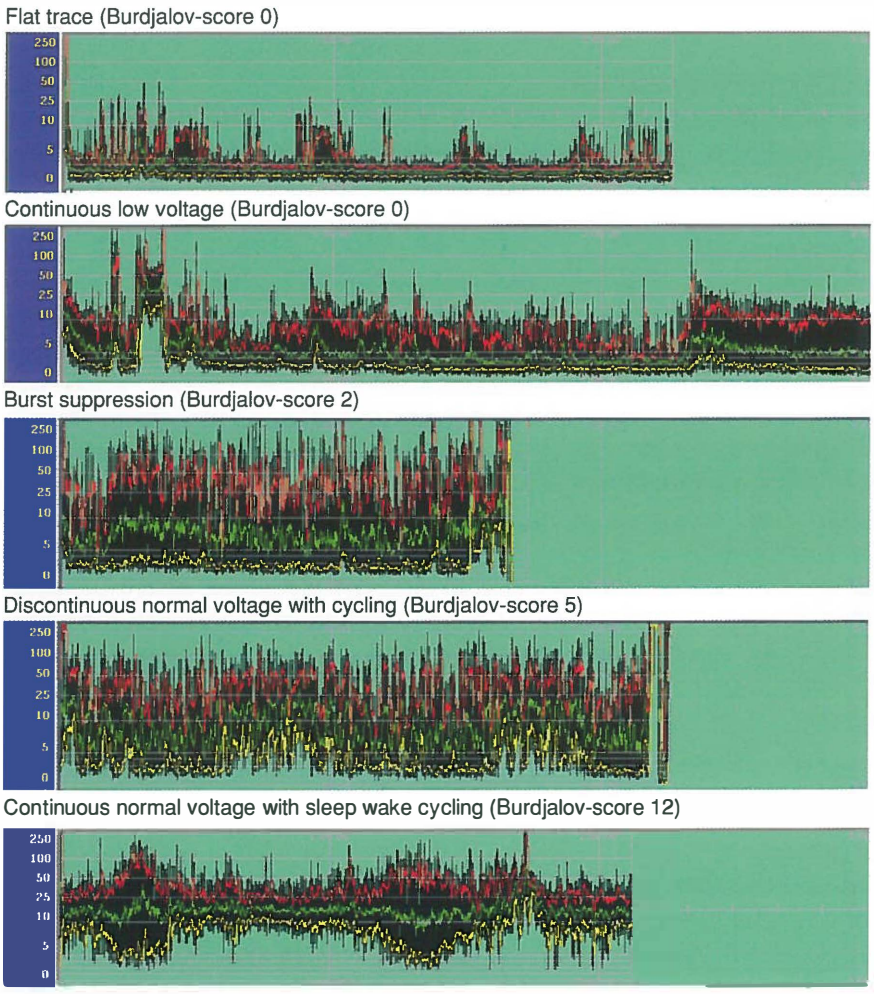

Figure 1: Examples of aEEG background patterns Amplitude centiles in yellow (5th) green (50th) and red (95th)

We assessed the aEEGs by pattern recognition and by calculating the centiles of the aEEG amplitudes.

During aEEG recording the nursing staff took note of any handling of the infant, clinical seizures, and administration of anticonvulsant or sedative drugs.

Assessing the aEEGs

Pattern recognition

Continuity, cycling, lower border, and bandwidth of the aEEG amplitude were determined. From these items the Burdjalov score was calculated (8). This score distinguishes three categories for the bandwidth: low span (< 15 μV), moderate span (15-20 μV), and high span (> 20 μV). As such we determined the bandwidth, taking the semi-logarithmic scale of the aEEG into account.

The aEEG amplitude centiles

In order to obtain additional quantitative measures, we calculated the mean of the 5^{th}, 50^{th}, and 95^{th} centiles of the aEEG amplitudes for the duration of the recording period each day.

Statistical analysis

We used the SPSS software for Windows, version 14.0 (SPSS Inc. Chicago, Illinois) for all analyses. For testing the correlation between the Burdjalov scores and the aEEG amplitude centiles the Pearson Rank Order Correlation coefficient was calculated and tested two-tailed. The variables that were tested for their relationship with aEEG amplitude centiles were: SNAP-II, 5 minute Apgar score, gestational age, mode of ventilation, treatment with caffeine, treatment with inotropes, and surfactant instillation. We performed a multivariate linear regression analysis to find the most significant model explaining the dependent variables. Variables entered the model at a significance level of $p < 0.1$. The Variance Inflation Factors (VIF) for the independent variables were calculated to measure the impact of collinearity among the variables in the model. A VIF above 2.5 was considered to indicate multicollinearity. The relationships were further analysed by calculating Pearson Rank Order Correlation coefficients. A p-value of < 0.05 was considered statistically significant.

Results

Study group

Data were collected on 43 infants of whom five were excluded: two due to large IVHs, and for three the data on the illness severity model were incomplete. The study cohort therefore consisted of 38 infants. Their gestational ages ranged from 26 to 31.9 weeks (mean 29.7 weeks, SD 1.4). We obtained 190 recordings. Artefacts were present in 49 recordings. The remaining 151 reliable recordings had a mean duration of 130 minutes. Surfactant was administered to 22 infants. In 15 infants the surfactant was given on the day of the aEEG recording. During 44 aEEG recordings infants were being ventilated mechanically and nasal continuous positive airway pressure (CPAP) or nasal intermittent mandatory ventilation (IMV) was given during 82 aEEG recordings. During the remaining 25 recordings infants received low flow. All infants but one could be weaned off the ventilator within four days. Eight infants were treated with inotropes because of low blood pressure. In our department infants receive

inotropes to maintain blood pressure if their mean arterial blood pressure in mm Hg drops below the gestational age in weeks despite volume administration. During treatment with inotropes the blood pressure is measured by means of an indwelling arterial catheter. During 12 recordings infants were receiving inotropes. During only two recordings morphine was administered. In our department we do not routinely sedate infants during artificial ventilation. Morphine is only administered prior to scheduled intubation and not prior to intubation in the delivery room. One infant received phenobarbitone because of clinical seizures. A summary of the clinical data of the study population is given in Table 1.

Table 1: patient characteristics (N= 38)

Gestational age, mean (SD), wk	29.7 (1.4)
Birth weight, mean (SD), g	1340 (380)
Male/female, n/n	14/24
Apgar score 5 min, mean (range)	7.8 (0-10)
Apgar score 5 min ≥ 7, n (%)	31 (82)
SNAP-II, mean (SD)	13.5 (7.8)
IVH	
No, n (%)	34 (89)
grade I, n (%)	2 (5)
grade II, n (%)	2 (5)
Initial ventilation	
nasal CPAP/IMV, n (%)	12 (32)
artificial ventilation, n (%)	26 (68)
Surfactant, n (%)	22 (58)
Inotropic support, n (%)	8 (21)

SNAP-II: score for neonatal acute physiology; IVH: intraventicular haemorrhage; CPAP: continuous positive airway pressure; IMV: intermittent mandatory ventilation.

The aEEG measurements

The aEEG amplitude centiles in relation to background patterns

The 5th and 50th amplitude centiles correlated significantly with the Burdjalov score (r= 0.71, $p < 0.0001$ and r= 0.52, $p < 0.0001$, respectively) (Figure 2).

The relationship between the SNAP II score and the aEEG amplitude centiles

The SNAP-II scores correlated negatively with the 5th and 50th aEEG amplitude centiles (r= -0.34, p< 0.0001 and r= -0.27, p= 0.001, respectively). These negative correlations were the strongest on the first day of life (r= -0.55, p= 0.005 and r= -0.47, p= 0.018, respectively). These correlations were no longer present after the fourth day.

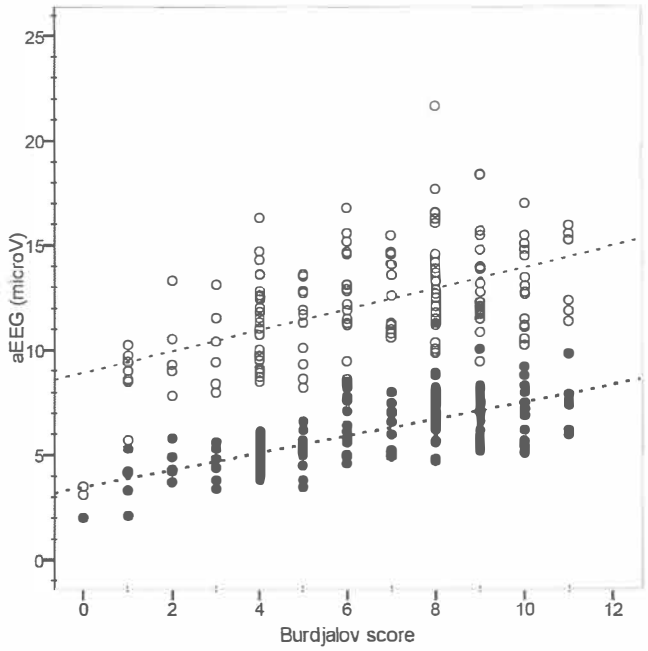

Figure 2: Relationship between Burdjalov-score and 5th and 50th aEEG amplitude centiles. ●: 5th amplitude centile; ○: 50th amplitude centile

Table 2: aEEG amplitude centiles for each postnatal day

	Postnatal age, days (N)				
	1 (25)	2 (36)	3 (27)	4 (31)	5 (32)
5th centile (µV), mean (SD)	5.7 (1.8)	5.9 (1.8)	6.2 (1.2)	6.3 (1.6)	6.1 (1.4)
50th centile (µV), mean (SD)	11.9 (3.5)	11.7 (3.1)	12.9 (2.2)	12.4 (2.4)	12.0 (2.2)
95th centile (µV), mean (SD)	36.9 (12.6)	38.7 (11.3)	40.2 (7.9)	39.0 (10.7)	34.1 (9.6)

N: number of aEEG recordings; SD: standard deviation.

Gestational and postnatal age

The amplitude centiles correlated significantly with the gestational age. 5th and 50th amplitude centile had a positive correlation with the gestational age (r= 0.40, $p < 0.0001$ and r= 0.16, $p = 0.05$ respectively). In contrast the 95th amplitude centile correlated negatively with the gestational age (r= -0.39, $p < 0.0001$).

The amplitude centiles did not change with increasing postnatal age. The amplitude centiles are shown in Table 2.

The five minute Apgar score

During the first two days of life the 5th and 50th aEEG amplitude centiles correlated positively with the Apgar score at 5 minutes (r= 0.37, p= 0.003 and r= 0.42, p= 0.001, respectively). This correlation was the strongest on the first day of life (r= 0.46, p= 0.022 and r= 0.50, p= 0.011, respectively).

Blood pressure

The 5th amplitude centile was significantly lower when inotropes were administered to maintain blood pressure (4.2 vs. 6.2 µV, $p < 0.001$). The 50th amplitude centile was also significantly lower during the administration of inotropes (9.4 vs. 12.4 µV, $p = 0.001$).

Mode of ventilation, surfactant instillation, and caffeine

Compared to infants who received nasal CPAP or nasal IMV, infants who received low flow via nasal cannula had significantly higher 5th aEEG amplitude centiles (p= 0.011). The 5th and 50th aEEG amplitude centiles were lower in infants receiving mechanical ventilation, compared to infants on low flow (p= 0.012 and p= 0.06, respectively). We found no significant differences between infants receiving nasal CPAP/IMV and infants receiving mechanical ventilation (Table 3). In addition, the 5th and 50th amplitude centiles were lower in infants who received surfactant on the day of the aEEG recording (p= 0.057 and p= 0.014, respectively).

Table 3: aEEG amplitude centiles in relation to ventilatory support

	aEEG amplitude centile (µV)		
	5th	50th	95th
LF, mean (SD)	6.9 (1.3)	12.9 (2.6)	36.3 (8.3)
CPAP/nIMV, mean (SD)	6.0 (1.4)	12.3 (2.3)	38.5 (10.3)
MV, mean (SD)	5.6 (1.9)	11.4 (3.2)	37.2 (12.2)

LF: low flow; CPAP: continuous positive airway pressure; nIMV: nasal intermittent mandatory ventilation; MV: mechanical ventilation.

Of the infants that were not mechanically ventilated during aEEG recording (N= 107), caffeine was administered on the day of aEEG recording in 48 (45%) cases. None of the infants received their loading dose during aEEG recording. The 5th aEEG amplitude centile of the infants who were receiving caffeine was significantly lower (p= 0.002). The 95th aEEG amplitude centile was higher in infants who were

receiving therapy with caffeine (p= 0.051). These effects disappeared when the lower gestational age was taken into account.

Apart from the gestational age, Apgar scores, blood pressure support, and ventilatory status, we found no correlations for any other clinical condition.

Epileptic activity
Two infants had epileptic activity, it appeared in three (2%) of the aEEGs. The epileptic discharges were all single seizures. aEEG amplitude centiles and Burdjalov scores were not different between aEEGs with and without epileptic activity. None of the infants experienced clinical seizures.

Morphine and anti epileptic drugs
Morphine was given during three recordings in two individual infants. There was no difference in aEEG amplitude centiles between these three recordings and the remaining recordings.

One infant received phenobarbitone during two aEEG recordings. The aEEG centiles were significantly lower during treatment with phenobarbitone ($p < 0.001$). This particular infant experienced severe birth asphyxia and already had an extremely abnormal background pattern (FT) prior to treatment with phenobarbitone.

Multivariate linear regression
Since the individual variables were likely to be interdependent we performed a multivariate linear regression analysis. Linear regression analyses were performed to examine the determinants of the 5th, 50th, and 95th aEEG amplitude centiles separately. We entered the variables gestational age, birth weight, SNAP-II score, 5 minute Apgar score, mode of ventilation, surfactant instillation, and use of inotropes into the model. When building a model, the 5th aEEG amplitude centile was best

Table 4: Summary of most significant model for predicting aEEG amplitude centiles

	R^2, constant	ß	95% CI	p-value	VIF
5th aEEG centile	0.22, -1.7				
GA		0.28	0.11 - 0.46	0.002	1.07
SNAP-II		-0.043	-0.076 to -0.009	0.012	1.25
Inotropes Y/N		-1.39	-2.27 to -0.51	0.002	1.18
50th aEEG centile	0.10, 13.1				
SNAP-II		-0.65	-0.13 to -0.004	0.036	1.18
Inotropes Y/N		-2.30	-3.96 to -0.64	0.018	1.18
95th aEEG centile	0.13, 125				
GA		-2.95	-4.19 to -1.72	<0.0001	1

aEEG: amplitude integrated EEG; CI: confidence interval; GA: gestational age; N: no; SNAP-II: score for neonatal acute physiology; VIF: variance inflation factor; Y: yes.

predicted by gestational age, use of inotropes, and the SNAP-II score. The 50th aEEG amplitude centile was best predicted by use of inotropes, and the SNAP-II score. The 95th aEEG amplitude centile was only predicted by gestational age. The model is summarised in Table 4.

Discussion

This study demonstrated that calculating aEEG amplitude centiles was a viable method for assessing aEEGs. Since the majority of preterm infants had a discontinuous background pattern at the start of aEEG recording, we assessed the aEEGs by pattern recognition, using the score published by Burdjalov et al. (8). In previous studies, aEEG patterns were only assessed by pattern recognition: analysing the lower margin of the amplitude, the appearance of continuity, and the presence of cycling of the background pattern. Burdjalov et al. designed a new scoring system based on these criteria. They demonstrated that infants with higher gestational ages have higher scores, indicating a more continuous and mature background pattern. We found a strong relationship between aEEG amplitude centiles and the assessments based on pattern recognition. The correlations we found between aEEG amplitude centiles and the Burdjalov scores were strong. Recently quantitative output data as the upper and lower margin amplitude of the aEEG tracings were reported (20). These output data are more or less comparable to the aEEG amplitude centiles that we used. We have chosen to use the 5th and 95th centiles and not upper and lower margin, because by using centiles it is clearly defined what the data represent. In our opinion, using amplitude centiles is a promising method for evaluating the electro-cerebral activity of preterm infants. With the evolution of CFM equipment on-line calculation of the characteristics of the aEEG (e.g. amplitude centiles) becomes available. This may lead to a better understanding of the aEEGs of preterm infants. The method will allow us to gain insight into the impact of certain clinical conditions on electro-cerebral activity (e.g. low blood pressure, sepsis, etc.).

Our second aim was to investigate whether the aEEGs of preterm infants were influenced by severity of illness. We found that during the first five days of life the aEEG amplitude centiles correlated negatively with SNAP-II, indicating a negative effect of severe illness on electro-cerebral activity. This is in line with data on fullterm infants, where severely asphyxiated infants have severely depressed background patterns (12, 13). This effect of illness severity was the most apparent during the first day of life and was no longer present at the fifth day of life. In addition, we found a positive correlation with the Apgar score at 5 minutes during the first two days of life. This effect was not caused exclusively by the fact that the more severely ill infants merely had a lower gestational age. Illness severity and gestational age predicted the electro-cerebral activity independently.

In addition to the depression of electro-cerebral activity with increasing severity of illness, the electro-cerebral activity was lower in infants with low blood pressure, as indicated by treatment with inotropes. This occurred irrespective of gestational age and illness severity. In case of loss of auto-regulation of cerebral blood flow, low blood

pressure may immediately result in lower cerebral perfusion. Auto-regulation is impaired in sick preterm infants (21, 22). As a result impaired cerebral perfusion may negatively influence electro-cerebral activity (23, 24). We speculated that, to some extent, the lower electro-cerebral activity we found in the more severely ill infants was caused by impaired cerebral perfusion.

Gestational age also influenced the aEEG amplitude centiles independently. Irrespective of the SNAP-II scores and the use of inotropes we found a positive effect on the 5th aEEG amplitude centile. The 5th aEEG amplitude centile increased with increasing gestational age. Concurrent with the increase of the 5th aEEG amplitude centile there was a decrease of the 95th centile with increasing gestational age. The decrease of the distance between the 5th and 95th amplitude centiles resulted in a raised lower border and a narrower bandwidth of the aEEG amplitudes. This finding was consistent with previous data (7, 8, 25, 26). These findings possibly reflect the maturation of brain activity with increasing gestational age. The higher peak electro-cerebral activity at a lower gestational age, expressed by the 95th centile, might be explained by the excitatory effect of GABA in preterm infants, in contrast to the inhibitory effect in fullterm infants (27).

Conventional EEG studies have demonstrated a shortening of inter burst interval (IBI) with increasing gestational age (5). With increasing gestational age more continuous activity will appear, eventually leading to the emergence of a tracé continue. The increase of more continuous activity will lead to an increase of the lower margin of the amplitude, as expressed by the 5th amplitude centile. A decrease of discontinuity will lead to a lower peak activity, as is expressed by a lower 95th amplitude centile

Another variable on which the SNAP-II score was based is the pO_2/FiO_2 ratio. A lower pO_2/FiO_2 ratio leads to a higher SNAP-II score. Respiratory distress syndrome might lead to a lower pO_2/FiO_2-ratio. Surfactant instillation and ventilatory support are often needed to treat respiratory distress syndrome. We found a negative effect of surfactant instillation on electro-cerebral activity. To a certain extent this may have contributed to the depression of electro-cerebral activity in more severely ill infants. This effect of surfactant was reported previously. In general it is transient, and disappears within one hour after instillation (28). In addition to surfactant instillation, we investigated the effect of the mode of ventilatory support on electro-cerebral activity. Very ill infants receive artificial ventilation more frequently and ventilation in itself may depress electro-cerebral activity. The electro-cerebral activity of infants who received nasal CPAP/IMV or artificial ventilation was lower in comparison to infants who received low flow. This lower activity could be explained by the lower gestational age (6, 8). Indeed, the difference in electro-cerebral activity between infants who received mechanical ventilation and surfactant treatment disappeared when gestational age and the use of inotropes were taken into account. Ventilation by itself did not play a major role in influencing electro-cerebral activity.

Recently it was reported that the aEEG amplitude and percentage of continuity

increased after i.v. administration of caffeine (29). We did not record aEEG during administration of the loading dose. The aEEG amplitude centiles of infants receiving maintenance treatment with caffeine on the day of aEEG recording were different: the 5th aEEG amplitude centile was lower, and the 95th aEEG amplitude centile was higher. These differences were opposite to the effects described by Supcun et al. (29) and may be explained by the difference in gestational age. This indicates that the instantly increased electro-cerebral activity attributed to caffeine is limited to the i.v. administration of the loading dose of caffeine. Accelerated maturation of electro-cerebral activity at a post menstrual age of 34 to 36 weeks has been attributed to treatment with aminophylline (30). We were not able to analyse this in our sample, since we only recorded aEEG during the first 5 days after birth.

During the first five days of life we found no change of the aEEG amplitude centiles. Other researchers reported an increase in discontinuous and continuous normal voltage patterns during the first weeks of life (9, 31). In these studies aEEG recordings were performed at weekly time intervals, or twice weekly at most, and the first recording was performed at a variety of postnatal ages. The strength of our study is that we performed daily recordings from the day of birth onwards, and therefore could evaluate day to day changes. Our data indicated that the increase in continuity of aEEG patterns, reported by others, did not occur during the first five days of life.

There were some limitations to our study. To date, long-term follow-up has not been analysed. Thus far, our study showed that aEEGs were influenced by several factors during the first days of life. These factors should be accounted for when studying the prognostic significance of aEEGs in preterm infants. Long-term follow-up will be an integrated part of future studies investigating the usefulness of early aEEGs for the prediction of long-term outcomes. The SNAP-II scores are based on criteria of the first day after birth. No scoring system is currently available that is appropriate for following the changes in clinical condition during early postnatal life. The CFM we used is not commercially available at this moment. Nevertheless the characteristics of this digital CFM are similar to the original CFM published by Maynard et al (19).

Conclusions

The assessment of aEEGs by calculating amplitude centiles proved to be a valid method. Electro-cerebral activity of preterm infants was influenced negatively by severe illness. This effect is the most apparent on the first day after birth. We found that two aspects of severe illness, i.e. a low 5 minute Apgar score, and low blood pressure had a suppressive effect on aEEG activity. Finally, electro-cerebral activity was clearly affected by gestational age.

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CHAPTER 6

The relationship between electro cerebral activity and cerebral oxygenation in preterm infants of less than 32 weeks of gestation

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Submitted

Abstract

Impaired cerebral perfusion and oxygenation may cause cerebral damage in preterm infants. At lower levels of cerebral perfusion and oxygenation electro-cerebral activity is disturbed. Cerebral oxygenation can be measured by near-infrared spectroscopy (NIRS) and electro-cerebral activity can be measured by amplitude-integrated EEG (aEEG). Our aim was to determine the relationship between regional cerebral tissue oxygen saturation (r_cSO_2), fractional tissue oxygen extraction (FTOE), and aEEG. We recorded longitudinal digital aEEG and r_cSO_2 prospectively in 46 preterm infants (mean GA 29.5 weeks, SD 1.7) for two hours on the 1st to 5th, 8th, and 15th day after birth. We excluded infants with germinal matrix hemorrhage exceeding grade I and recordings of infants receiving inotropes. FTOE was calculated using trans-cutaneous arterial oxygen saturation ($tcSaO_2$) and r_cSO_2 values: ($tcSaO_2$-r_cSO_2)/$tcSaO_2$. aEEG was assessed by calculating the mean values of the 5th, 50th, and 95th centiles of the aEEG amplitudes. The aEEG amplitude centiles changed with increasing gestational age. FTOE and aEEG amplitude centiles increased significantly with postnatal age. More mature electro-cerebral activity was accompanied by increased FTOE. FTOE also increased with increasing postnatal age and decreasing hemoglobin levels.

Introduction

Preterm infants are at risk of developing intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) (1), two conditions that may lead to permanent cerebral damage. Impaired cerebral oxygenation may contribute to the development of both IVH and PVL and may thus contribute to the development of permanent cerebral damage (2). We defined oxygenation of the brain as oxygen delivery to the brain, which depends on cerebral perfusion and arterial oxygen content. Impaired oxygen delivery may also cause cerebral damage, independently of IVH and PVL.

The balance between cerebral oxygenation and oxygen use can be monitored by near-infrared spectroscopy (NIRS) (3). This is a non-invasive method that measures regional cerebral oxygen saturation (r_cSO_2). R_cSO_2 reflects the oxygen saturation in a mixed vascular bed dominated by venules. Fractional tissue oxygen extraction (FTOE) can be calculated from r_cSO_2 and transcutaneous arterial oxygen saturation ($tcSaO_2$) (4). It reflects the balance between oxygen supply and oxygen consumption and may thus indicate cerebral hypoxemia or ischemia.

At lower levels of cerebral perfusion and oxygenation electro-cerebral activity is disturbed. Amplitude-integrated EEG (aEEG) is a marker of electro-cerebral activity. In term infants aEEG can be severely abnormal following perinatal hypoxia-ischemia (5). In preterm infants electro-cerebral activity is generally discontinuous and changes with gestational and postnatal age (6,7). With increasing gestational and postnatal age continuous electro-cerebral activity increases.

Little is known about the relationship between electro-cerebral activity and cerebral oxygenation in relatively healthy preterm infants. Therefore the aim of our study was to investigate the relationship between r_cSO_2, FTOE, and aEEG. We hypothesized that increased electro-cerebral activity will lead to higher oxygen consumption and, as a result, to higher FTOE, as long as it is not accompanied by increased oxygen delivery.

Methods

For this prospective observational study we initially selected 50 preterm infants that had been admitted to the neonatal intensive care unit of the University Medical Center Groningen between May 2006 and July 2007. All preterm infants with a gestational age of 26 to 32 weeks admitted on their first day after birth were eligible for inclusion. We excluded infants with major chromosomal or congenital abnormalities. After the initial selection we excluded three infants from further analysis that had developed an IVH exceeding grade I according to Volpe (8). We also excluded eleven recordings of infants that required inotropes to maintain blood pressure at the time of the aEEG and NIRS recordings. The final study cohort consisted of 46 infants. Written informed consent was obtained from both parents. The study was approved by the review board of the University Medical Center Groningen.

The aEEG and r_cSO_2 were measured simultaneously within the first 24 hours after birth and subsequently on the 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 8^{th}, and 15^{th} day.

The aEEG measurements

We used a digital cerebral function monitor (CFM) that was not commercially available at the time of the study. It consisted of an amplifier connected to a laptop computer that contained software for digital aEEG processing. In addition to displaying the aEEG pattern it also displayed the original EEG. The device recorded the aEEG through two neonatal electrocardiogram (ECG) electrodes with a diameter of 15 mm (Neotrode II, Conmed, Utica, NY, USA). The electrodes were placed in P3 and P4 position (international 10-20 system). The common electrode was placed conveniently anywhere on the infant's body. We used a digital direct current (DC) common average reference amplifier (Porti-X by TMSi, Enschede, the Netherlands) comprising a high input impedance (> 2 GΩ) and a 22 bits sigma-delta Analog to Digital Converter with a resolution of 0.0715 μV per bit. The electrodes were connected to the amplifier by means of shielded cables to prevent electrical noise and alternating current (AC) power interference pick-up. Loss of electrode contact was sensed by the amplifier's input circuitry and signaled to the data acquisition software. Low (< 0.5 Hz) and high frequencies (> 25 Hz) were attenuated by first order high and low pass filtering. The EEG was stored on a hard disc and the aEEGs were processed.

The aEEG processor was constructed in software and comprised a signal shaping filter, a semi-logarithmic rectifier, a peak detector, and a smoothing filter. Its characteristics were similar to the CFM constructed and described by Maynard (9), and to all commercially available machines. All values were filtered by box-car averagers with a time window of 60 seconds. To obtain additional information the mean of the aEEG amplitude and the mean peak and trough values were computed and displayed. The mean trough and mean peak values represented the 5th and 95th centiles of the aEEG amplitudes. An example of an aEEG recording, which also displays the aEEG amplitude centiles, is shown in Figure 1.

We assessed the aEEGs by pattern recognition and by calculating the centiles of the aEEG amplitudes.

While the aEEGs were being recorded the nursing staff noted down any handling of the infant, clinical seizures, and administration of anticonvulsant or sedative drugs.

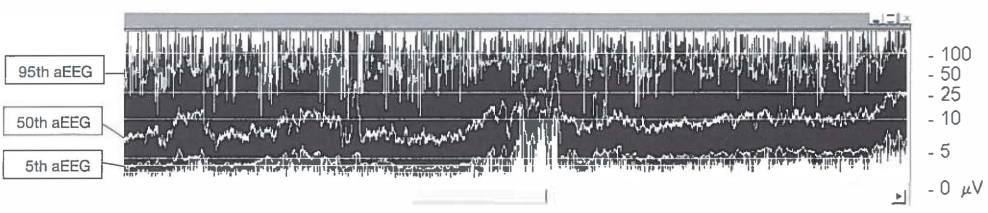

Figure 1: Example of a display of the digital recorded aEEG. 5th aEEG: 5th centile of the aEEG amplitude; 50th aEEG: 50th centile of the aEEG amplitude; 95th aEEG: 95th centile of the aEEG amplitude.

Pattern recognition
Different background patterns were distinguished according to Hellström-Westas (10). Background patterns were characterized as: continuous normal voltage (CNV), discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage or flat trace.

The presence or absence of sleep-wake cycling (SWC) and the occurrence of epileptic activity was also noted down. SWC was recognized as cyclical variations in the bandwidth of the aEEG trace indicating cycling of sleep stages.

The aEEG amplitude centiles
In order to obtain additional quantitative measures we calculated the mean of the 5th, 50th, and 95th centiles of the aEEG amplitude for the recording period on each day. Artifacts were identified, and confirmed with the use of the raw EEG, after which they were excluded from quantitative analysis.

Near-infrared spectroscopy
R_cSO_2 was measured with the INVOS 4100 near-infrared spectrometer (Somanetics Corporation, Troy, MI) in combination with the pediatric SomaSensor. This technology is based on the fact that biological tissue is relatively transparent to near-infrared light (600 to 900 nm). The optical sensor measures the quantity of reflected light photons as a function of two wavelengths (730 and 805 nm) and determines the spectral absorption of the underlying tissue (11,12). NIRS differentiates oxygenated hemoglobin from deoxygenated hemoglobin that has distinct absorption spectra. The ratio of oxygenated hemoglobin to total hemoglobin reflects the regional oxygen saturation of tissue. The SomaSensor has two detectors at a distance of 3 and 4 cm from the near-infrared optode. The detector placed at 3 cm from the optode receives light scattered predominantly from scalp and skull. The detector placed at 4 cm receives light scattered from scalp, skull, and cerebral tissue. Thus, by subtraction, the two detectors measure the oxygen saturation in the underlying cerebral tissue.

R_cSO_2 was measured over a two-hour period. Fifteen minutes were allowed for the measurement to stabilize. The optical sensor was placed to the left frontoparietal side of the infant's head and held in place by elastic bandaging.

Simultaneously, we measured transcutaneous arterial oxygen saturation ($tcSaO_2$) by pulse oximetry. We calculated FTOE with the equation FTOE = ($tcSaO_2$-r_cSO_2)/ $tcSaO_2$.

Statistical analysis
SPSS software for Windows, version 16.0 (SPSS Inc. Chicago, Illinois) was used for all analyses. Because of normal distribution differences in centiles of the aEEG amplitude between certain types of background patterns were analyzed by Student-t test. Results were expressed as mean values ± SD. The Pearson correlation coefficient (two-tailed) was calculated to test the correlation between FTOE, r_cSO_2, and the

centiles of the aEEG amplitude. The variables that were tested for their relationship with the aEEG amplitude centiles and FTOE were postnatal age, gestational age, mean arterial blood pressure, hemoglobin level, and arterial partial pressure of carbon dioxide ($PaCO_2$). To test whether clinical data were different between subgroups of our cohort (e.g. infants with and without SWC), we used the Mann-Whitney U test for continuous variables, and Fisher's Exact test for categorical variables. Finally, we performed a multivariate linear regression analysis to find the most significant model explaining FTOE. Variables entered the model at a significance level of $p < 0.1$. A p value of < 0.05 was considered statistically significant.

Results

Study group

Data were collected on 46 infants whose gestational ages ranged from 26 to 31.9 weeks (mean 29.4 ± 1.7). We obtained 238 combined recordings of aEEG and r_cSO_2. The mean daily recording time was 128 minutes (SD 31). Twenty-nine infants needed artificial ventilation for initial stabilization and all but one received surfactant. During 55 recordings infants were mechanically ventilated and nasal continuous positive airway pressure (CPAP) was given during 98 recordings. During the remaining 86 recordings infants either received low flow via nasal canula or they had no respiratory support. The majority of infants could be weaned off the ventilator within five days. Because we do not routinely sedate infants during artificial ventilation none of the infants received morphine during the study period. Clinical data of the study population are summarized in Table 1.

Table 1: Patient characteristics (N= 46)

Gestational age, mean (SD), wk	29.5 (1.7)
Birth weight, mean (SD), g	1311(390)
Antenatal steroids, n (%)	44 (96)
Apgar score 5 min, mean (range)	7.4 (3-10)
Apgar score 5 min ≥ 7, n (%)	35 (76)
MABP, mean (SD), mm Hg	37 (6.8)
IVH	
No, n (%)	44 (96)
grade I, n (%)	2 (4)
Initial ventilation	
low flow, n (%)	1(2)
nasal CPAP/IMV, n (%)	16 (35)
artificial ventilation, n (%)	29 (63)
Surfactant, n (%)	28 (61)
Hb, mean (SD), mmol/l	8.7 (1.6)
$PaCO_2$, mean (SD), kPa	5.5 (0.9)

MBAP: mean arterial blood pressure; IMV: intermittent mandatory ventilation; Hb: haemoglobin level.

Pattern recognition

During the entire study period the aEEG traces of the majority of infants showed the discontinuous background patterns BS or DNV (Table 2). From the 5th day after birth the frequency of continuous patterns increased (Chi² for trend; p= 0.036). SWC was present in some infants from the first day after birth and increased on the 3rd postnatal day (Chi² for trend; p= 0.003). Postmenstrual age (gestational age + postnatal age) was significantly higher when SWC was present (30.3 vs 29.7 weeks, p= 0.023). Postnatal age was also significantly higher when SWC was present (6.0 vs 4.6 days, p= 0.015).

Table 2: frequencies of aEEG background patterns and sleep wake cycling in relation to postnatal age

	Postnatal day						
aEEG n (%)	1	2	3	4	5	8	15
BS	6 (23)	7 (19)	4 (12)	3 (8)	4 (12)	1 (3)	0
DNV	17 (65)	26 (70)	25 (73)	30 (81)	26 (74)	27 (77)	23 (66)
CNV	3 (12)	4 (11)	5 (15)	4 (11)	5 (14)	7 (20)	12 (34)
SWC (%)	8 (31)	20(54)	26 (76)	26 (70)	24 (69)	23 (66)	27 (77)

SWC already appeared on the first day after birth. No differences were found in gestational age, birth weight, and Apgar scores between infants with and without SWC on the first day after birth. Infants without SWC were significantly more often treated with surfactant (p= 0.026), and more often artificially ventilated (p= 0.073).

The different background patterns had significantly different aEEG amplitude centiles (Table 3). In comparison to DNV, BS had a significantly lower mean 5th ($p < 0.001$) and 50th amplitude centile ($p < 0.001$). In comparison to CNV, the mean 5th and 50th centiles of BS were also significantly lower ($p < 0.001$) and the mean 95th centile was higher (p= 0.011). In comparison to CNV, DNV had significantly lower mean 5th and 50th amplitude centiles ($p < 0.001$) while the mean 95th amplitude centile was higher (p= 0.002).

Table 3: aEEG amplitude centiles in relation to background patterns

	Amplitude centiles (µV)		
aEEG (n) mean, SD	5th	50th	95th
BS (40)	3.9, 0.94	9.1, 1.7	37, 7.8
DNV (174)	6.1, 1.5	12, 2.6	36.5, 10.7
CNV (25)	8.1, 1.6	14.2, 3	32, 7.2

The aEEG amplitude centiles

There was a change of electro-cerebral activity with both postnatal age and gestational age. The 5th amplitude centile correlated positively with both postnatal age (r= 0.19, p= 0.004) and gestational age (r= 0.56, $p < 0.001$). We found the opposite effect on the 95th amplitude centile: both postnatal age (r= - 0.14, p= 0.037) and gestational age (r= - 0.19, p= 0.003) had negative correlations with the 95th amplitude centiles.

We found no significant changes in the aEEG amplitude centiles during the first five days after birth. The 5th amplitude centile increased significantly between the 5th and 15th day (student-t, p= 0.001) and between the 8th and 15th day (student-t, p= 0.021). The changes in the aEEG amplitude centiles are shown in Figure 2.

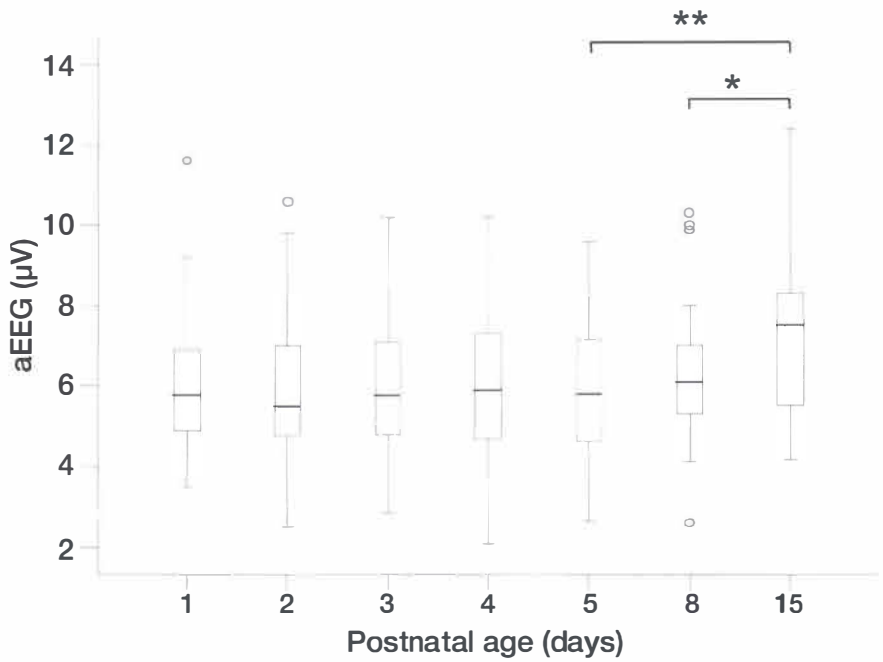

Figure 2: The relationship between the 5th aEEG amplitude centile and postnatal age. *: p< 0.05; **: p < 0.01

The relationship between R_cSO_2, FTOE, and postnatal age

FTOE changed with postnatal age. R_cSO_2 decreased (r= - 0.27, $p < 0.001$), whereas FTOE increased with postnatal age (r= 0.32, $p < 0.001$).

We found no changes in r_cSO_2 and FTOE during the first five days after birth. After the 5th day there were significant changes in r_cSO_2 and FTOE. R_cSO_2 decreased from 79 percent on the 5th day to 76 percent on the 8th day and 70 percent on the 15th

day. The differences between the 5th and 15th day and the 8th and 15th day were significant ($p < 0.001$ and $p = 0.007$, respectively). While r_cSO_2 decreased there was an increase in FTOE from 0.16 to 0.20 between the 5th and 8th day ($p = 0.03$) and from 0.20 to 0.26 between the 8th and 15th day ($p = 0.013$). The increase between the 5th and 15th day was highly significant ($p < 0.001$). The course of FTOE is shown in Figure 3.

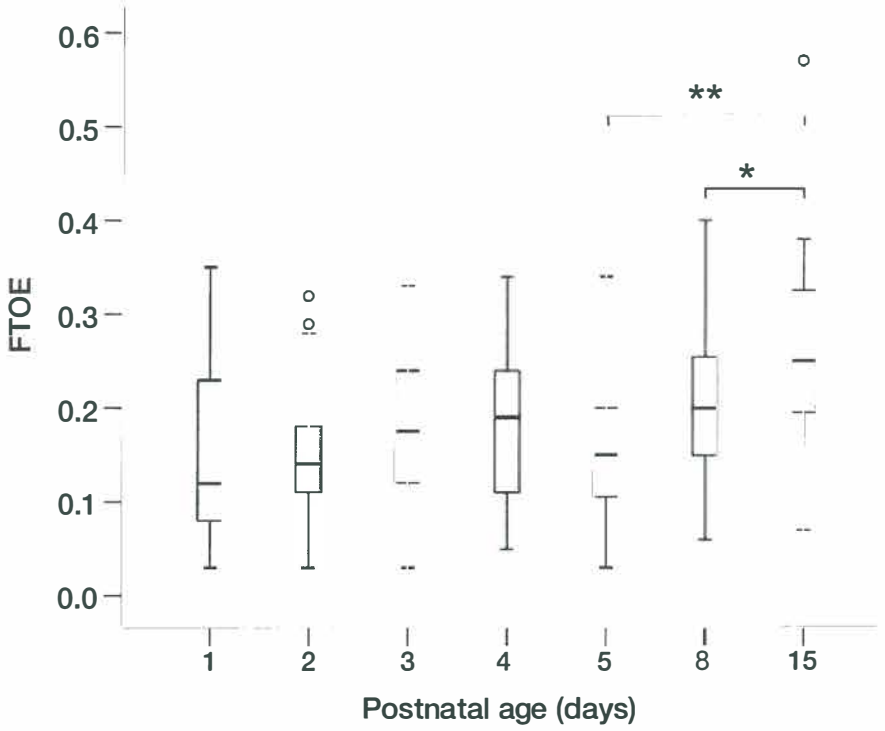

Figure 3: The relationship between FTOE and postnatal age.
*: p< 0.01; **: p < 0.001

The relationship between aEEG amplitude centiles and FTOE
The 5th and 50th aEEG amplitude centiles correlated positively with FTOE ($r = 0.26$, $p < 0.001$ and $r = 0.14$, $p = 0.035$, respectively). The 95th amplitude centile correlated negatively with FTOE ($r = -0.13$, $p = 0.042$).

The relationship between FTOE and the clinical variables
Since several clinical conditions may interfere with cerebral oxygenation and perfusion we investigated whether these conditions confounded the relationships we found for FTOE and aEEG amplitude centiles. We checked blood pressure, persistent ductus ateriosus (PDA), ventilatory support, $PaCO_2$, and hemoglobin levels. There

was no relationship between FTOE and blood pressure or $PaCO_2$. We did, however, find a negative correlation between hemoglobin levels and FTOE (r= -0.30, p= 0.001). FTOE was influenced by the mode of ventilation. We found that infants on low flow or infants without ventilatory support had higher FTOE compared to infants on nasal CPAP or artificial ventilation (0.22 vs. 0.16, p < 0.001). The values of $tcSaO_2$ were significantly higher in infants on low flow or infants without ventilatory support compared to infants with CPAP (97% vs. 94%, p < 0.001) and artificial ventilation (97% vs. 91%, p < 0.001). There was no difference between infants on nasal CPAP or artificial ventilation. There was a slightly lower FTOE in case of a PDA during recording (0.17 vs. 0.19, p= 0.029). The hemoglobin levels were not different between the infants with and without a PDA.

Multivariate linear regression

Since individual variables are likely to be interdependent we performed a multivariate linear regression analysis to examine the determinants of FTOE. The variables we entered into the model were aEEG amplitude centiles, postnatal age, hemoglobin level, mode of ventilation, and PDA. The 5th aEEG amplitude centile (ß: 0.12 (95% CI: 0.003 - 0.20); p= 0.01), 95th aEEG amplitude centile (ß: -0.002 (95% CI: -0.003 – 0); p= 0.022), postnatal age (ß: 0.005 (95% CI: 0.001 - 0.009); p= 0.009), and hemoglobin level (ß: -0.011 (95% CI: -0.021 to -0.001); p= 0.030) remained in the model, explaining 22.5% of the variance.

Discussion

Our study demonstrated a clear relationship between electro-cerebral activity and FTOE. We found increased FTOE with changing electro-cerebral activity. Increase of the 5th aEEG amplitude centile, decrease of the 95th aEEG amplitude centile and, consequently, a narrower bandwidth of the aEEG were associated with higher FTOE. This higher FTOE may indicate higher cerebral oxygen consumption.

Electro-cerebral activity changed with increasing gestational and postnatal age. The 5th aEEG amplitude centile increased while the 95th amplitude centile decrease concurrently. We consider this narrower bandwidth of the aEEG to be a more mature background pattern. We observed the same maturational effects on aEEG with increasing postnatal age. From the 5th day onwards a larger proportion of aEEGs showed continuous normal voltage. The aEEG amplitude centiles also changed significantly after the 5th postnatal day. Several previous publications reported maturational effects of both postnatal and gestational age on electro-cerebral activity (7,13,14). Mostly, these studies recorded aEEG at weekly intervals. Our study indicated that this change in electro-cerebral activity generally took place in the second week after birth.

From the 5th postnatal day onwards the maturation of electro-cerebral activity occurred simultaneously with an increase of FTOE, an observation we reported previously (15). It has been reported that increased cerebral oxygen consumption in case of increased metabolism is met by an increase of cerebral blood flow (16), the

so-called neurovascular coupling. In that case FTOE is expected to remain stable. Similar as Yoxall et al (16), we found no increase of FTOE during the first week after birth. Instead, we found an increase of FTOE during the second week after birth, which was independent of hemoglobin levels and more mature electro-cerebral activity. We speculate that this higher FTOE is at least partly the result of increased oxygen consumption, due to increased metabolism. It is an established fact that metabolism in preterm infants nearly doubles after the first week after birth (17). Theoretically, the increase of FTOE could also be the result of impaired oxygen delivery due to decreased cerebral blood flow. In case of lower cerebral blood flow, however, we would expect a decrease in electro-cerebral activity. Moreover, none of the infants were treated with inotropes and their blood pressure was within the normal ranges. We found no relationship between FTOE and blood pressure. This suggests that within normal ranges of blood pressure cerebral auto-regulation is intact

Independent of electro-cerebral activity and postnatal age we found an increase of FTOE with decreasing hemoglobin concentration. If the hemoglobin concentration decreases the absolute amount of oxygen transported is decreasing. Within ranges of constant oxygen demand this may lead to a higher extraction of oxygen (18,19). These results are in line with the study of Roche-Labarbe et al. (20). They found an increase in cerebral oxygen consumption during the first six weeks after birth, which was related to a decrease of hemoglobin over the same period. The infants in our study cohort were relatively healthy preterm infants. If an infant's clinical condition worsens oxygen supply can become critical. Under such circumstances maintaining the hemoglobin level can become crucial in preserving oxygen supply to the brain, and thus preventing brain damage.

FTOE was influenced by the mode of ventilatory support. Infants that were artificially ventilated or treated with nasal CPAP during aEEG recordings had lower FTOE than infants that only required low flow or no support at all. This was, however, not independent of other factors like aEEG amplitude centiles and postnatal age. Oxygen delivery to the brain was not impaired in infants without artificial ventilation or nasal CPAP, $tcSaO_2$ was even higher in infants on low flow via nasal canula. Therefore, in hemodynamically stable infants cerebral oxygenation is not influenced by the severity of respiratory distress syndrome and the mode of ventilation itself. This is in line with a previous study that compared cerebral oxygenation in preterm infants with and without respiratory distress syndrome (21). If, however, respiratory failure is accompanied by circulatory insufficiency this may lead to impaired cardiac output and, consequently, to lower cerebral perfusion. Lower cerebral perfusion may lead to higher FTOE and to a change of electro-cerebral activity.

A negative effect on electro-cerebral activity was reported following the so-called "InSurE" procedure for the treatment of respiratory distress syndrome (22). This negative effect was attributed to the administration of opioids prior to intubation. With this opoid induced change in electro-cerebral activity cerebral oxygenation and FTOE were constant, suggesting a decreased oxygen demand in case of decreased

electro cerebral activity. Since none of the infants in our study received opioids, changes in electro-cerebral activity in the present study reflect differences in postnatal and gestational age.

We checked for other potentially confounding factors such as CO_2 level and PDA. Hypocarbia causes cerebral vasoconstriction that results in decreased cerebral blood flow. Decreased cerebral blood flow may result in increased FTOE. A recent study reported a negative correlation between transcutaneous PCO_2 and FTOE (23). By contrast, we found no correlation between FTOE and $PaCO_2$ level. One of the differences was that we did take blood samples, either arterial or capillary, to measure $PaCO_2$ level. The $PaCO_2$ levels were within the normal ranges. It may well be possible that at lower CO_2 levels cerebral blood flow diminishes and that FTOE increases as a result. Wardle et al found an increase in FTOE with a decrease of $PaCO_2$ levels (24). They found an effect of $PaCO_2$ levels within infants. We did not perform repeated and combined measurements of $PaCO_2$ levels with FTOE. Therefore, it is possible that we have missed temporal changes of FTOE in relation to $PaCO_2$ levels. We did find a lower FTOE in case of a PDA during recording. Because several factors were likely to be interdependent we performed a multivariate analysis. A PDA did not contribute to FTOE independently. Lower FTOE seems to be explained by a lower gestational and postnatal age in infants with a PDA. Infants with a lower gestational age had less mature electro-cerebral activity, which was associated with lower FTOE. The difference in FTOE could not fully be explained by a difference in hemoglobin levels.

In conclusion, this study demonstrated a tight relationship between electro-cerebral activity and oxygen consumption. As electro-cerebral activity matured, oxygen consumption increased. Other factors influencing oxygen consumption were postnatal age and hemoglobin level. This combination of FTOE and electro-cerebral activity may be a useful biomarker of brain function in high-risk infants. The combination of high FTOE and low electro-cerebral activity may well reflect impairment of cerebral oxygenation or perfusion and may be an indication for clinicians to focus on preserving oxygen supply to the brain in order to limit brain damage. More research is needed to study the implications of this combined monitoring approach for treating infants during the neonatal period.

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